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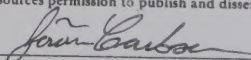
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Title and subtitle HEPATIC TUMOR GROWTH. Influence of hepatic artery occlusion. An experimental study in rats.			
Abstract In order to study the influence of hepatic arterial interruption on hepatic tumor growth, an experimental model was designed in rats. By inoculation of a defined suspension of viable tumor cells directly into the liver parenchyma, a solitary, well-defined, ellipsoid and easily measurable tumor was obtained. The ellipsoid shape was independent of tumor size and tumor type. The tumors could be measured with vernier calipers under laparotomy and at angiography at the greatest (a) and smallest (b) diameters. Estimation of tumor volume of unremoved liver tumors was obtained using the formula $V = a \times b^2/2$ thus making it possible to study the tumor growth in living animals. Hepatic arteriography revealed a transient increased arterial vascularization by increasing tumor size. Portography showed portal vessels leading to the central part of the tumor. Hepatic artery ligation (HAL) was found to reduce the growth of three different experimental liver tumors as compared to controls. This effect was not observed after liver dearterialization, i.e. separating the liver from all surrounding structures except the hepatic veins, common bile duct and the portal vein. The effect in reducing hepatic tumor growth after HAL seemed to be dependent on the tumor size at the time for the HAL procedure. Embolization of the hepatic artery with Spongostan gave a similar though transient reduction in liver tumor growth. In order to further reduce the hepatic tumor growth whole body microwave hyperthermia was applied in combination with HAL. This combination gave no further reduced hepatic tumor growth compared with only HAL. When hyperthermia was given the same day and one day after HAL the mortality was 50 %.			
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SOLITARY EXPERIMENTAL LIVER TUMORS IN RATS.

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SUMMARY

Experimental solitary ellipsoid liver tumors in the rat can be induced by inoculation into the liver parenchyma of a tumor cell suspension of known potency. The liver tumor can be measured in vivo on the surface of the liver during laparotomy as the greatest (a) and the smallest (b) superficial diameter. With the size of 15-700 mm³ tumor volume can be accurately calculated according to the formula $V = a \times b^2 / 2$ compared with the actual volume from the extirpated tumors. This makes it possible to estimate the growth rate of unremoved liver tumors in rats.

Key words : Liver tumors; tumor measurements.



In a malignant tissue cells have escaped from the restrictions and controls of normal growth. The malignant growth process develops from a dynamic, frequently changing and complex series of events. There is no single parameter better than tumor growth rate that can give information on cell population and effect of different therapeutic manoeuvres on tumor growth (Lala 1971).

A previously induced tumor transplanted in inbred animals is a convenient model for tumor experiments even if growth characteristics and histological findings change during repeated transplantation (Begg 1971; Moore and Dixon 1977).

An experimental model for studying the changes in tumor volume of therapeutic manipulations on an unremoved liver tumor in rats would be most valuable.

Inducing liver tumors by a hepatocarcinogenic substance or as a cell suspension of tumor cells injected into the portal vein in rats is accompanied by scattered tumor growth in the liver (Nilsson and Zettergren 1967; Fisher and Fisher 1959a). An increased number of injected tumor cells is followed by an increased tumor take in the liver (Fisher and Fisher 1959a). Other factors affecting tumor take and growth rate are, for example, surgical trauma to the liver (Fisher and Fisher 1959b), repeated laparotomies (Fisher and Fisher 1959c), alteration of liver blood flow (Fisher and Fisher 1961a; Fisher and Fisher 1963), nutrition (Fisher and Fisher 1961b), anticoagulants (Fisher and Fisher 1961c) and reticuloendothelial interference (Fisher and Fisher 1961d; Fisher and Fisher 1962).

Scattered tumor growth in the liver is a disadvantage in many experiments as estimations of tumor size need examination of the entire liver after removal (Nilsson and Zettergren 1967; Fisher and Fisher 1959a).

The changes in tumor size between subsequent repeated measurements express the effect of therapeutic manipulations. The most suitable experimental model is one that enables accurate repeated tumor size estimations in living animals.

Most solid tumors in animals and man grow as three-dimensional aggregates and in most tissues the tumors appear spheroid or ellipsoid in shape (Willis 1968).

Estimation of tumor size can be done from measurements of the tumor in one (Mayneord 1932; Marsh 1933) or two (Brues et al 1939; Mottram 1935) dimensions by vernier calipers.

In a superficially growing tumor repeated measurements of the product of the greatest and smallest diameters express a good description of tumor growth, provided that the tumor does not change its average shape under the observation period (Steel,

Adams and Barrett 1966).

Calculation of tumor volume is, however, best done if the tumor is measured in three dimensions. Measurements of three diameters are easy to do if the tumor is situated superficially. This can be done with different formulas (Dethlefsen et al 1968; Schrek 1935; McCredie et al 1965; Hermens and Barendsen 1967) which gave a good agreement with in vitro measurements (Schrek 1935). With central growth in an organ it is almost impossible to make accurate measurements in vivo in all three dimensions.

The aim of the present study was to design a standard and reproducible method to produce solitary liver tumors in the rat using a tumor cell suspension as inoculum and to investigate if measurements in two dimensions of the visible superficial surface of the unremoved liver tumor could serve as an accurate estimation of the tumor volume compared with the measurement in three dimensions of the dissected and extirpated tumors or tumor weight.

MATERIAL AND METHODS

Thirty-four inbred Wistar rats of both sexes with a weight of 180-240 g were used in the experiment. They were maintained on a standard pellet diet and water ad libitum. The tumor used in the study was a N-methyl-N-nitrosoguanidine-induced adenocarcinoma of the colon, transplanted into the kidney under sterile conditions every 10th day (Steele and Sjögren 1974).

The cell suspension used in the experiment was manufactured by dissecting the tumor from the kidney and then cutting it into small fragments. Trypsin-EDTA solution and normal rat serum were added and mixed. This procedure was repeated three times. Then the test tube was centrifuged and the supernatant removed. The trypsinization was stopped by inactivated rat serum. After adding trypan blue the vital cells were counted in a Blichner chamber (Boyse et al 1962). The final cell solution was then diluted to a suspension containing 1.0×10^6 viable tumor cells per 0.1 ml.

Within one hour after the preparation, 0.1 ml of the tumor cell suspension was inoculated into the periphery of the central lobe of the liver just under the liver capsule. Access to the liver was achieved through a midline abdominal incision under ether anesthesia. In order to avoid leakage of tumor cells from the injection site after withdrawal of the injection needle a Spongostan sponge was pressed against the opening until hemostasis was complete.

Within varying intervals up to three weeks the abdominal cavity was reopened and the tumors were measured with vernier calipers in the greatest (a) and smallest (b) superficial visible diameters on the ventral side of the liver lobe.

The tumors were then extirpated and by macroscopic dissection freed from normal liver tissue. Measurements with vernier calipers were then made of the same diameters as before, expressing the actual greatest (c) and smallest (d) diameters and, finally, the third diameter (e) was measured. The tumor weight was recorded in mg.

Mathematical and statistical calculations

From the two superficial measurements in vivo the tumor size was calculated using four different formulas (Steel, Adams and Barrett 1966; Kopper and Steel 1975; Chambers and Scott 1930; Simpson-Herren and Lloyd 1970):

$$V_1 = a \times b$$

$$V_2 = \frac{\pi}{6} \left(\frac{a \times b}{2} \right)^3$$

$$V_3 = a \times (b)^2 / 2$$

$$V_4 = (\sqrt{a \times b})^3$$

The three measurements from the extirpated tumors were used to calculate the tumor volume by the formula (Dethlefsen et al 1968):

$$V_5 = \frac{\pi}{6} \times c \times d \times e$$

The calculated tumor sizes were then compared with each other and with the tumor weight. Student's t-test and regression analyses were used as statistical methods.

RESULTS

No animals showed any signs of malnutrition during the experiments. No mortality was registered. At autopsy no macroscopic signs of metastases were found. The extirpated tumors were in all animals, except one, ellipsoid in shape. The smallest actual tumor volume calculated according to V_5 was 15.4 mm³ and the largest 710.3 mm³.

Calculations using the formulas V_1 - V_5 gave the following results (Table 1).

Logarithmic mean tumor size calculated from two superficial measurements according to V_2 and V_3 showed no statistically significant difference from V_5 ($p > 0.10$). The other formulas (V_1 and V_4) gave highly significant differences from formula V_5 ($p < 0.0001$).

Formula	Mean ($\pm$ SD)	Logarithmic mean ($\pm$ SD)
V_1	57 ($\pm$ 37)	3.880 ($\pm$ 0.62)
V_2	263 ($\pm$ 241)	5.197 ($\pm$ 0.94)
V_3	219 ($\pm$ 175)	5.062 ($\pm$ 0.89)
V_4	483 ($\pm$ 425)	5.820 ($\pm$ 0.93)
V_5	223 ($\pm$ 163)	5.128 ($\pm$ 0.83)

Table 1. Mean tumor size and geometric mean tumor size for different formulas.

The standard deviation of the paired difference of logarithmic mean tumor volume between V_5 and V_3 was smallest (Table 2).

Compared formulas	Standard deviation
$V_1 - V_5$	0.282
$V_2 - V_5$	0.265
$V_3 - V_5$	0.226
$V_4 - V_5$	0.247

Table 2. Standard deviation of paired difference between the formulas.

Regression analyses on logarithmic values resulted in $V_5 = 0.572 + 0.900 \times V_3$, $R^2 = 0.938$ (Fig 1 and Table 3).

$$\begin{array}{ll}
 V_5 = 0.092 + 1.298 \cdot V_1 & R^2 = 0.935 \\
 V_5 = 0.713 + 0.849 \cdot V_2 & R^2 = 0.927 \\
 V_5 = 0.572 + 0.900 \cdot V_3 & R^2 = 0.938 \\
 V_5 = 0.092 + 0.865 \cdot V_4 & R^2 = 0.935
 \end{array}$$

Table 3. Regression analysis on logarithmic values for the different formulas.

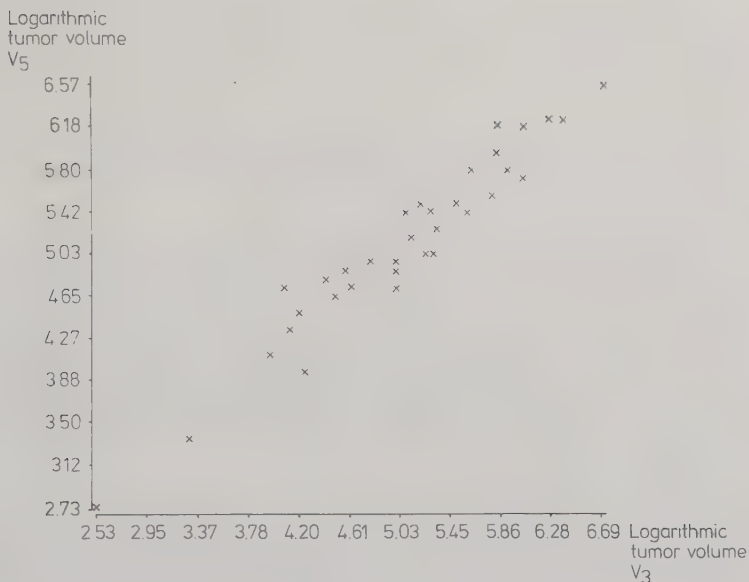


Fig 1. Regression analysis for formulas V_3 and V_5 .

Calculation according to V_3 represented the best fit. The regression coefficient (0.900) being closest to 1 and the proportion of explained variation (R^2) was highest (Table 3). The correlation between logarithm of tumor volume according to V_3 and logarithm of weight was the highest ($r = 0.88$) and the same correlation according to V_5 was 0.94 (Table 4).

Formula	Correlation (r)
V_1	0.87
V_2	0.87
V_3	0.88
V_4	0.87
V_5	0.94

Table 4. Correlation between logarithmic tumor volume and logarithmic tumor weight.

The optimal formula found in this investigation for calculation of tumor volume from two superficial measurements is $V_6 = a^{1.05} \times b^{1.62} \times 0.999$.

DISCUSSION

To gain better insight into the treatment of liver tumors in man it is necessary to have a suitable and reproducible in vivo animal model for the study of clinical inquires.

By injecting a cell suspension with a known number of viable tumor cells in the periphery of the central lobe of the liver in the rat it was possible to produce solitary, ellipsoid liver tumors. There was no difference in the shape of the tumors whatever their size. Calculation of the tumor volume from superficial measurements of two visible tumor diameters in vivo gave different results depending on which formula was used. The most accurate formula seemed to be $V_3 = a \times b^2 / 2$ when compared with the tumor volume calculated from the measurements in three diameters of dissected and extirpated tumors.

The model may therefore be most suitable for use in studies following the influence of different therapeutic maneuvers on unremoved liver tumors in the rat with the size of 15-700 mm³. Each measurement needs, however, a laparotomy which may influence the tumor growth (Fisher and Fisher 1959c).

ACKNOWLEDGEMENTS

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VASCULARIZATION AND TUMOR VOLUME ESTIMATIONS OF SOLITARY LIVER
TUMORS IN RATS.

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ABSTRACT

Tumor vascularity of a transplantable N-methyl-N-nitrosoguanidine induced adenocarcinoma of the colon in the liver was investigated by repeated hepatic arteriography and portography. The arterial vascularity was influenced by tumor size; a transient increase in vascularization was observed when tumor size increased. Portography revealed portal vessels concentrated to the peripheral parts but some portal vessels were also observed leading to the central part of the adenocarcinomas. The tumor volume of solitary adenocarcinomas in the liver could be estimated from measurements of the largest (a) and smallest (b) diameters on the angiogram according to the formula $V = a \times b^2/2$.

KEY WORDS: liver tumors; arteriography; portography; tumor measurements.

INTRODUCTION

An anatomical investigation with corrosion preparations by Segall in 1923 showed vessels in human liver tumors originating from the hepatic artery (1). Other injection methods and arteriographic investigations have later confirmed this observation (2, 3, 4, 5). Based on this, hepatic artery ligation (HAL) has been used in patients with unresectable liver malignancy in order to interrupt the blood supply to the tumors and to induce tumor necrosis (6, 7, 8).

Injection methods, arteriography and portography of the liver in experimental animals show the same vascular pattern of the tumors as in humans, with predominant arterial vessels to the tumors (2, 9, 10, 11, 12, 13). The existence of predominantly arterial vessels is not affected whether the liver tumors are induced by hepatocarcinogenic substances, tumor transplants or injection of tumor cells. The vascular pattern has been seen to vary with the histopathological tumor type and tumor size. Arterial vessels are seen in cholangiocarcinoma independent of size and in small transplanted tumors (3-7 mm). Hepatocellular carcinoma and larger transplanted tumors have both arterial and portal vessels, with the latter concentrated to the periphery of the tumors (9, 11, 12). With injection of dye solutions portal vessels have been identified sporadically within an implanted Walker tumor (14).

There are varying results of blood flow measurements in liver tumors.

With ¹³³Xenon technique liver tumors in humans are shown to be less perfused than normal liver tissue (15, 16). With microsphere technique it is shown in rats that liver tumors are less or similarly perfused with arterial blood as normal liver parenchyma (17, 18). But other blood flow measurements with RISA and microspheres have shown that liver tumors are better perfused with blood than normal liver parenchyma (10, 11, 19, 20). Measurements of the blood flow to small liver tumors of less than 0.3 g using the RISA and microsphere techniques reveal that the blood flow originates both from the hepatic artery and portal vein (10, 11). When the tumor increases in size the arterial blood supply becomes predominant (10, 11) and there is a simultaneous decrease in vascular bed and relative tumor blood flow with increasing size (19).

Repeated tumor measurements is one method for the evaluation of the effect on tumor growth of different therapeutic procedures. The volume of unremoved liver tumors can be accurately calculated according to the formula $V = a \times b^2 / 2$ (a = largest diameter, b = smallest diameter (21). The visible diameters can be measured during laparotomy with vernier calipers. This formula can be used provided that the ellipsoid shape of the liver tumors remains constant during tumor growth (22).

Surgical trauma such as repeated laparotomies stimulates the growth rate of liver tumors (23). Angiography can outline experimental liver tumors in rats thus enabling measurements of tumor size without laparotomy (24).

The present experimental study was performed in order to investigate the vascular pattern of an experimental adenocarcinoma in the liver by arteriography and portography, to study changes in the arterial vascular pattern with increasing size and, finally, to estimate tumor volume by angiography.

MATERIAL AND METHODS

Thirteen inbred male Wistar rats weighing 200-250 g were used. They were maintained on a standard rat pellet diet and water ad libitum.

Under ether anesthesia and through a midline incision 0.1 ml of a transplantable N-methyl-N-nitrosoguanidine-induced adenocarcinoma (NGW) in the colon was inoculated as a tumor cell suspension containing 1.0×10^6 viable tumor cells into the periphery of the central lobe of the liver (21).

Hepatic arteriography was performed according to the previously described technique with the modification that all repeated arteriographies were carried out via the inguinal incision (25). Portography was performed with a catheter placed in the portal vein introduced via the superior mesenteric vein during laparotomy. Arteriograms and portograms were obtained in the lateral projections.

Tumor measurements were performed during laparotomy with vernier calipers at the largest (a) and the smallest (b) visible superficial diameters on the ventral side of the central liver lobe. Measurements were also made at the largest (c) and the smallest (d) diameters on the film after correction for the magnification of 3 %.

Ten, 14 and 21 days after tumor cell inoculation coeliac angiography was carried out in nine rats under ether anesthesia followed by tumor measurements during laparotomy. The surviving animals subjected to repeated angiographies were sacrificed on the 21st day. Portography was performed in four rats 14 days after tumor cell inoculation, then the animals were sacrificed.

The vascular pattern of the tumors was estimated from the arteriograms and portograms.

The tumor volume was calculated with the formula $V = a \times b^2/2$ (A) in vivo and $V = c \times d^2/2$ (B) on the film. The tumor volume was transformed to logarithmic values. Regression analyses were used in order to compare volumes A and B.

RESULTS

Three of nine rats subjected to coeliac angiography died, two at the first and one at the second angiography. The cause of death was in all cases due to perforation of the aorta by the catheter. Only the surviving six rats subjected to tumor measurements on all three occasions were included in statistical analyses.

No macroscopic metastases were found at autopsy in any rat. No significant changes in weight of the animals were observed during the experimental period.

Angiography or portography revealed in all the rats a solitary ellipsoid tumor localized in the periphery of the central liver lobe. Angiography and portography revealed a well defined border between normal liver parenchyma and tumor in all rats. The ellipsoid shape of the adenocarcinomas at angiography and laparotomy remained constant during the experimental period.

Ten days after tumor cell inoculation (tumor diameter 3-8 mm), arteriography revealed a hypovascularized central part and a hypervascularized periphery of all the adenocarcinomas (Fig 1). Four days later the size of the tumor feeding artery had increased. In six of seven tumors (diameter 5-12 mm) the difference between the central part and the periphery was not so evident and the vascularization was more homogenous (Fig 2). Twentyone days after tumor cell inoculation (diameter 8-20 mm) the border between central and peripheral parts of the tumor became visible, the central parts were again hypovascularized and the arterial vessels to the periphery of the tumor had increased in size (Fig 3).

Fourteen days after tumor cell inoculation (tumor diameter 7-13 mm) the portography revealed portal vessels concentrated to the periphery but also some portal vessels leading to the central part of the tumor which appeared loaded with contrast (Fig 4).

Regression analyses of the two different measurements of tumor volume at day 10, 14 and 21 are shown in Fig 5. The correlation coefficient was 0.97 on the 10th day, 0.94 on the 14th day and 0.97 on the 21st day. The correlation coefficient of the total 18 occasions of tumor measurements under laparotomy and at angiography was 0.97 (Fig 5).

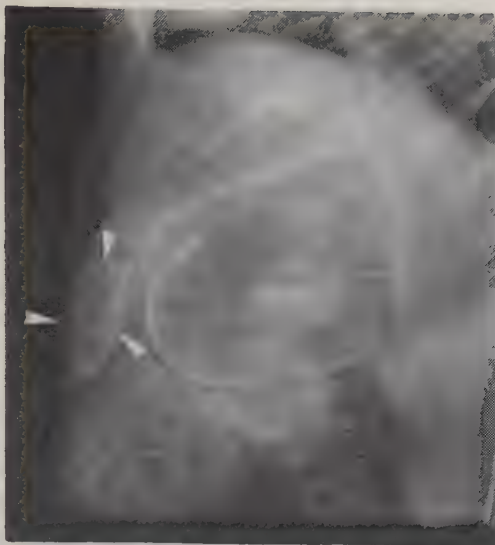


Fig 1. Coeliac angiography (late phase) showing single liver tumor 10 days after tumor inoculation with a hypovascularized central part and the hypervascularized periphery (tumor arrows).

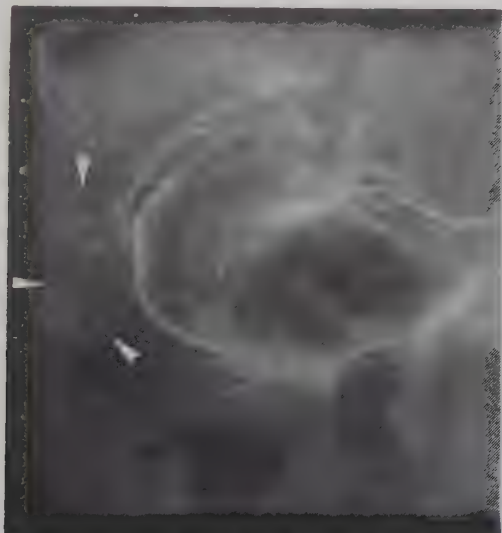


Fig 2. Second angiography four days later showing an enlarged tumor with a more homogeneous arterial blood supply and widening of the feeding artery (tumor arrows).

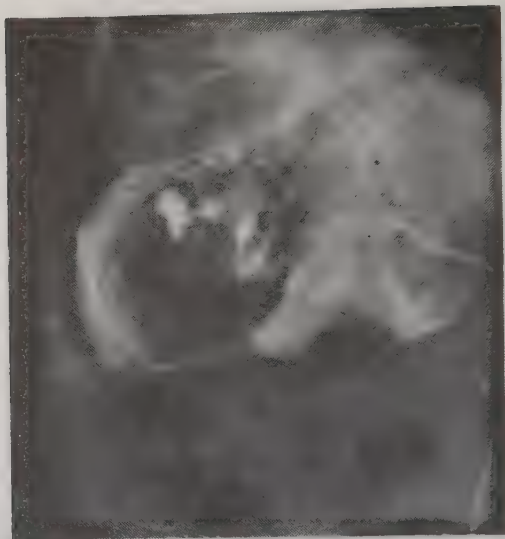


Fig 3. Angiography 21 days after tumor inoculation showing a hypovascularized central part with areas of contrast "pooling" and widening of the vessels in the periphery of the tumor.

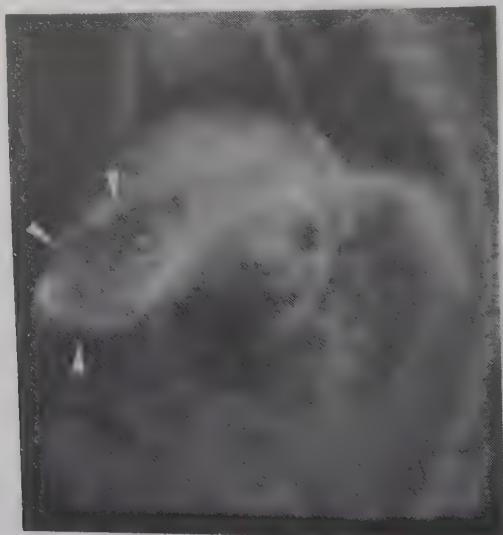


Fig 4. Portography demonstrating portal vessels in the periphery and the central part of the tumor (tumor arrows).

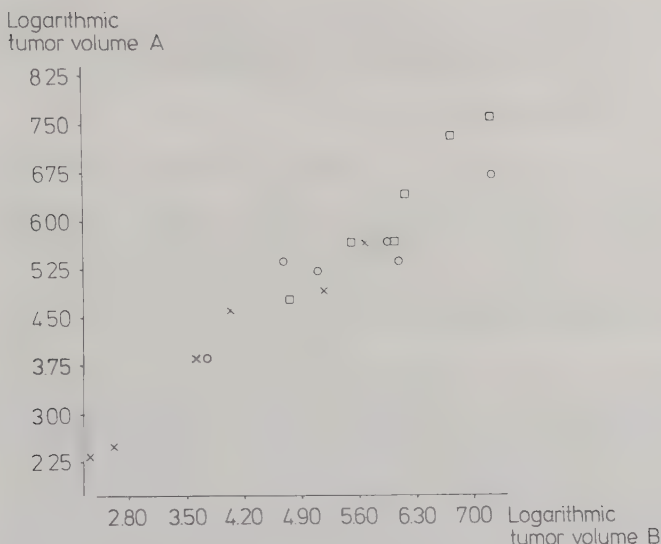


Fig 5. Regression analyses for the two different measurements of tumor volume at laparotomy (A) and at angiography (B). X on the 10th day, O on the 14th day and □ on the 21th day. Correlation coefficient $r = 0.97$.

DISCUSSION

Inoculation of a defined tumor cell suspension straight into the liver parenchyma of the rat induced solitary ellipsoid tumors which could be visualized by arteriography and portography. The margin between tumor and normal liver parenchyma was well defined.

Portal vessels were seen in the periphery but some vessels also seemed to reach the central part of the tumors as indicated by the finding of contrast loading in the central parts of the tumor. This suggests that after interruption of the arterial blood supply the portal circulation may nourish even central parts of certain liver tumors. Portal vessels and an increased portal blood supply to the periphery of liver tumors have been observed after hepatic artery ligation (11, 15) but except for the findings by Honjo and Matsumura (14) using injection of dye solutions and Nilsson and Zettergren (9) using portography there is no description of portal vessels in the central part of a liver tumor.

The arterial vascular pattern of the experimental liver tumors was related to tumor size which is in agreement with the findings by Ackerman (12). By injection of silicone rubber he found that small

liver tumors (1-2 mm) were supplied by vessels from both the portal vein and hepatic artery, and moderately sized tumors (3-7 mm) were predominantly supplied by arterial vessels. Larger tumors (7-33 mm) showed a great variation in the vascular pattern. They were mainly supplied by vessels from either the portal vein or the hepatic artery or by a combination of the two (12).

The relative arterial blood flow of the present tumor (NGW) has been investigated by Sundqvist et al (17). They found a decreasing relative arterial blood flow with increasing tumor size. There is thus no evident correlation between the arterial vascular pattern identified at angiography and arterial blood flow measured by the microsphere technique. This statement is supported by that angiography in the present study showed a transient period of increased arterial vascularization with increasing tumor size.

In patients with unresectable hepatoma and metastatic colon cancer Kim et al have analysed tumor vascularity as a prognostic factor (26). The prognosis after hepatic artery ligation and regional intraarterial infusion of chemotherapeutic agents seemed to be better the more vascularized the hepatic tumor appeared to be on the angiogram. The influence of tumor size on the tumor vascularity was not mentioned.

In a previous report it was found that measurements of the visible largest and smallest superficial diameters of an unremoved liver tumor gave a good estimation of the volume and could be used for calculation of growth rate provided that the shape of the tumor did not change during the observation period (21). This implies that repeated angiograms of a solitary liver tumor could be used for calculation of its growth rate. The permanent ellipsoid shape of the tumor found both on the surface of the liver and in the lateral view on the angiogram in this study verifies these findings.

It was also found that repeated tumor volume calculations from measurements in two dimensions of solitary liver tumors can be accurately performed by arteriography using the formula $V = a \times b^2 / 2$. This can avoid several laparotomies which stimulate liver tumor growth (23). No investigations have, however, been carried out on the possible effect of repeated angiography on liver tumor growth.

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INTERRUPTION OF HEPATIC ARTERIAL SUPPLY IN RATS WITH LIVER TUMORS

Running title: Interruption of hepatic arterial supply.

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KEY WORDS: hepatic artery ligation; liver tumors; liver dearterialization.

ABSTRACT

Liver tumors receive their main blood supply from the hepatic artery. Various types of interruption of arterial blood supply to the liver are considered to give a reduction in liver tumor growth. Inoculation of a tumor cell suspension of a transplanted benzo(a)pyrene-induced sarcoma was used to induce a tumor in the liver of Lister-hooded rats. The influence of hepatic artery ligation (HAL) and regular liver dearterialization, i.e. dividing of all structures to the liver except portal vein, common bile duct and hepatic veins, was tested against control and sham operation. Ligation of the hepatic artery gave a statistically significant temporary reduction of liver tumor growth. A continuous growth of the tumors was found after regular liver dearterialization, sham procedure and in the controls. Seven and 14 days after the procedures this difference between HAL and regular liver dearterialization was recognized statistically significant ($p < 0.001$) in groups with only five animals.

INTRODUCTION

In clinical practice hepatic artery occlusion or dearterialization of the liver has been used for the treatment of primary and secondary liver malignancy (Almersjö, Bengmark, Engevik, Hafström, Nilsson 1966; Almersjö, Bengmark, Rudenstam, Hafström, Nilsson 1972; Balasegaram 1972; Bengmark, Fredlund, Hafström, Vang 1974; Kondahl, Funding 1972; Nilsson 1966; Sparks, Mosher, Hallauer, Silverstein, Rangel, Passaro Jr, Morton 1975; Farndon 1977; Bengmark, Dahl, Fredlund 1979; Madding, Kennedy, Sogemeier 1970; Mays 1967). The procedure is still experimental and absolute indications have not been established.

The liver has a dual blood supply, the hepatic arteries and the portal vein. Most liver tumors, both primary and secondary, receive their main blood supply from the hepatic artery (Ackerman, Lien, Kondi, Silverman 1969; Breedis, Young 1954).

Hepatic artery occlusion for treatment of liver tumors is based on the principle of inducing tumor necrosis by means of ischemia-anoxia. The possibility exists that mechanisms other than anoxia can be responsible for the effects on liver tumors of this procedure.

Experiments with hepatic artery ligation in rats with liver tumors have resulted in induced necrosis in tumors, reduction in tumor growth and a higher rate of survival (Nilsson, Zettergren 1967; Nilsson, Rudenstam, Zettergren 1967; Carlsson, Ekelund, Hafström, Jonsson, Stigsson 1981). Interruption of liver artery results in necrosis of the tumor cells but normal liver parenchyma will only suffer minor damage because of nutrition from the portal vein (Madding, Kennedy, Sogemeier 1970; Mays 1967; Nilsson, Zettergren 1967; Nilsson, Rudenstam, Zettergren 1967; Fortner, Mulcare, Solis, Watson, Golbey 1973; Mori, Masuda, Miyanaga 1966). After hepatic artery occlusion the decrease in blood flow of the tumors is more pronounced than that in normal liver parenchyma (Taylor, Bennett, Sherriff 1979; Gelin, Lewis, Nilsson 1968). In clinical practice the effect on liver tumors is only temporary and no prolonged survival has been registered (Almersjö, Bengmark, Rudenstam, Hafström, Nilsson 1972; Bengmark, Fredlund, Hafström, Vang 1974).

Fast development of arterial collaterals to the liver is one explanation for the temporary effect of the discontinued hepatic arterial supply (Mickels 1953; Bengmark, Rosengren 1970; Koehler, Korobkin, Lewis 1975; Plengvanit, Chearani, Sindhvananda, Damrongsak, Tuchinda, Viranuvatti 1972). The periphery of the liver tumors receives its main blood supply from the portal vein and in this zone vital tumor tissue is found after hepatic artery ligation (Ackerman, Lien, Kondi, Silverman 1969; Breedis, Young 1954; Taylor, Bennett, Sherriff 1979). The numerous arterial collaterals to the liver and intrahepatic anastomoses between the arterial and

portal systems probably minimize the ischemic period in the tumor after occlusion of the hepatic artery (Mickels 1953). A possible increased portal flow through the liver tumors after hepatic artery occlusion may be another explanation for the temporary effect of hepatic artery interruption on liver tumor growth (Taylor, Bennett, Sherriff 1979).

There are many important questions to be studied concerning interruption of the arterial supply to the liver, e.g. what is the basic principle of the destructive effect on the malignant tumor, is there any possibility of improving the effect by irradiation, cytostatics or hyperthermia, can the normal liver parenchyma be protected from hypoxic damage? Other vital questions are whether the interruption of the arterial liver blood flow influences extra-hepatic cancer growth, if there are other techniques working on the same principle but giving a better effect (Hafström, Nobin, Persson, Sundqvist 1980; Hafström, Persson, Sundqvist 1980a; Hafström, Persson, Sundqvist 1980b).

The aim of the present study was to establish a standard and easily reproducible procedure for interruption of hepatic arterial supply in rats with liver tumors.

MATERIAL AND METHODS

Twenty inbred Lister-hooded rats of both sexes with a weight of 220-250 g were used. They were maintained on a standard pellet diet and water ad libitum.

Under ether anesthesia the abdominal cavity was opened through a midline incision. A transplantable benzopyrene-induced sarcoma was inoculated in the form of a cell suspension of 1.0×10^6 viable tumor cells per 0.1 ml in the periphery of the central lobe of the liver (Carlsson, Gullberg, Hafström 1982).

Ten days later the animals were again anesthetized with ether and the abdominal cavity was reopened. Using vernier calipers the tumors were measured on the ventral side of the central liver lobe at the greatest (a) and the smallest (b) superficial diameters. The tumor volume was calculated according to the formula $V = a \times b^2 / 2$.

The animals were then randomized into four groups. In group A five animals were controls and no manipulations with the liver were performed except for tumor measurements. In group B five animals had all structures in the hepatic duodenal ligament divided except for the portal vein, common bile duct and hepatic artery. A 6-0 silk suture was placed around the proper hepatic artery distal to the ramification of the gastro-duodenal artery but was not tied. The entire liver was freed from its surrounding attachments except for the hepatic veins. This procedure is referred to as sham operation. In group C five animals had the proper hepatic artery in the gastro-duodenal ligament isolated and ligated with 6-0

silk suture without dividing the structures in the hepato-duodenal ligament. The liver was not freed from its surrounding attachments (HAL). In group D five animals were exposed to the same procedure as in group B but the 6-0 silk suture was adequately tied - regular liver dearterialization.

Seven and 14 days after the procedure, the tumor was measured with vernier calipers under relaparotomies. On the 14th day the animals were sacrificed and autopsy carried out.

Statistical analyses were made on logarithmic values with Student's t-test and analyses of covariance.

RESULTS

There were no differences in mortality between the groups. Mortality occurred between day 17 and day 24 after tumor cell inoculation. One rat in group A, two in group B and one in group D died.

At autopsy 24 days after inoculation of the tumor cells there were no macroscopic signs of metastases in any animal. The tumors in the liver were during the whole experimental period ellipsoid in shape. No significant changes in weight of the animals in any cases were observed during the experimental period compared with the weight at day 0.

On the day of the experimental procedure (day 10) there were no statistically significant differences in tumor volumes between the groups (Fig 1).

Seven days later there was a statistically significantly smaller geometric tumor volume in the animals subjected to hepatic artery ligation (group C), compared with the other groups ($p < 0.0001$). The tumor volume in group A was statistically significantly smaller compared with groups B and D ($p < 0.002$). On the 24th day after the inoculation the tumor volume in group C was significantly smaller compared with the other groups ($p < 0.001$).

The changes in geometric mean tumor volume for the four groups during the experimental period are shown in Fig 1.

Analyses of covariance on day 17 adjusted for the tumor volume on day 10 showed a statistically significantly smaller increase for group C compared with the other groups ($p < 0.0001$) and group A ($p < 0.04$) compared with B and D. There was a significant difference between groups A and C with a smaller increase in group C ($p < 0.0001$).

The same analyses on day 24 showed a significantly smaller increase in group C compared with the other groups ($p < 0.001$).

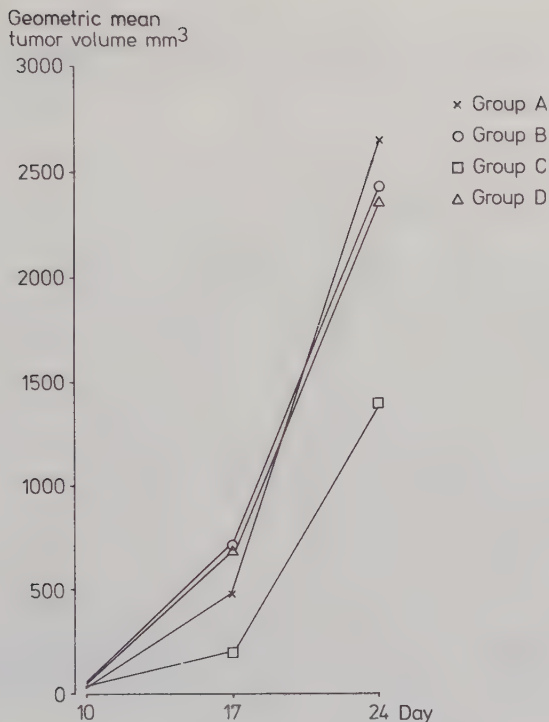


Fig 1. Geometric mean tumor volume for the groups during the experimental period.

DISCUSSION

It has not been proven that the effect on tumor growth after interruption of the arterial blood supply to the liver is due to the instituted anoxia-hypoxia in liver tumors.

Malignant tumors in the liver as well as elsewhere may have clones which are dependent on anaerobic metabolism, and anoxia-ischemia should not affect them. It is also usually anaerobic tumors which are more low-differentiated. In clinical practice HAL has been most valuable in treating carcinoid tumors (Aune, Schistad 1972; McDermott Jr, Hensle 1974; Sivula, Sipponen 1976). These tumors are highly differentiated, well vascularized and probably not anaerobic.

Because of its temporary effect, hepatic artery occlusion may not be a single procedure for treatment of liver tumors. Other methods,

synergistic or additive to the HAL procedure must be tested. In order to do this it is necessary to have a standard and reproducible method for hepatic artery occlusion. In clinical practice two major techniques are suggested (Almersjö, Bengmark, Hafström, Leissner 1976), hepatic artery ligation (HAL) and regular liver dearterialization of which the latter is a more extensive procedure.

In the present experimental study a slower tumor growth after hepatic artery ligation (HAL) than in the controls was verified up to the 14th day. Regular liver dearterialization or sham operation resulted in no reduction of the liver tumor growth. On the contrary, in these groups a faster tumor growth was observed up to the 7th day. These effects of regular liver dearterialization as well as the sham procedure may be due to extended surgical trauma of these procedures. Massive surgical trauma to the liver has been found to stimulate an increased liver tumor growth (Fisher, Fisher 1959).

Another explanation for the absence of the supposed effect on tumor growth of regular liver dearterialization in the present study may be that this is only observed within the first three or four days after the procedure.

The possibility also exists that the method for registering the effects of the procedure by means of tumor measurements with vernier calipers is too uncertain. This method of registering tumor growth is, however, well established (Carlsson, Gullberg, Hafström 1982; Steele, Adams, Barrett 1966) but the observed effect ought to be verified by, for instance, histopathological means.

Quantitative estimations of necrosis by microscopy are, however, too uncertain (Nilsson, Zettergren 1967; Nilsson, Rudenstam, Zettergren 1967). Another possibility for registering tumor growth is by an estimation of the release of polyamines or other tumor markers (Anehus, Bengtsson, Andersson, Carlsson, Hafström, Yngner, Heby 1981).

In conclusion hepatic artery ligation (HAL) seems to be a more accurate experimental procedure for a temporary reduction of liver tumor growth than regular liver dearterialization. This was verified with high statistical significance with only five animals in each group. HAL is a simple and reproducible method for further studies in this field.

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INFLUENCE OF HEPATIC ARTERY LIGATION ON LIVER TUMOR GROWTH IN RATS

Running title: Hepatic artery ligation for liver tumor growth.

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KEY WORDS: hepatic artery ligation; liver tumors.

ABSTRACT

Liver tumors, both primary and secondary, receive their main blood supply from the hepatic artery. Hepatic artery ligation (HAL) causes a reduction in tumor growth and tumor necrosis. In this experiment, three different experimental tumors were used to study the effect of hepatic artery ligation at different intervals after the occlusion of the arterial blood supply. Hepatic artery ligation causes a reduction in liver tumor growth for all three tumors but with some variation according to age and type of tumor.

INTRODUCTION

The survival of patients with liver malignancy, both primary and secondary, is restricted (10, 12) so any method improving the survival for these patients is of great clinical value.

Both primary and secondary tumors in the liver receive their main blood supply from the hepatic artery (1, 13). The dual blood supply to the liver enables interruption of the blood flow in the hepatic artery. Interruption of the arterial supply to the liver induces necrosis in liver tumors but only minor damage in normal liver tissue (3, 18, 25, 26, 27, 28). In clinical practice there is so far no study showing any definite benefit of this procedure.

Patients with the carcinoid syndrome are still the most suitable subjects for palliation with hepatic artery ligation (5, 19, 22, 23, 32).

It is postulated that the effect of hepatic artery ligation is governed by the principle of inducing tumor necrosis by means of ischemia - anoxia. A temporary reduction in liver tumor growth is observed after hepatic artery ligation (14, 16). This temporary effect may be due to the multiple collaterals to the liver which cause fast revascularization (11, 14, 21, 30, 31). Increased portal flow through the liver tumors after HAL is also a possible cause (35). A further possible mechanism for the temporary reduction in liver tumor growth after hepatic artery ligation is stimulation of anaerobic tumor cells by hypoxia. In a previously experimental study (16) regular liver dearterialization was tested versus HAL and it was shown that only HAL was a reproducible method which caused a reduction in liver tumor growth up to the 14th day after the ligation.

Although hepatic artery ligation is used in clinical practice (4, 9, 32) the method is still an experimental model and may not be a single procedure for control of liver malignancy. To improve the effect of hepatic artery ligation chemotherapy has been added but its utility has not yet been established (2, 7, 8, 18, 29, 34).

Other methods added to HAL for improved growth control of the liver tumors need further research.

Questions of importance in this report are when the effect of hepatic artery ligation is most pronounced and if the sensitivity to HAL is related to the type of the tumor.

The aim of the present study was to investigate the effect on the growth of different transplantable liver tumors at varying times after hepatic artery ligation.

MATERIAL AND METHODS

Forty-eight inbred Lister-hooded rats and 24 inbred Wistar rats of both sexes with a weight of 200-250 g were used. They were maintained on a standard pellet diet and water ad libitum.

Under ether anesthesia the abdominal cavity was opened through a midline incision. In all animals a tumor cell suspension of 1.0×10^6 viable tumor cells per 0.1 ml was inoculated into the central liver lobe (15). The design of the experiment is shown in Fig 1.

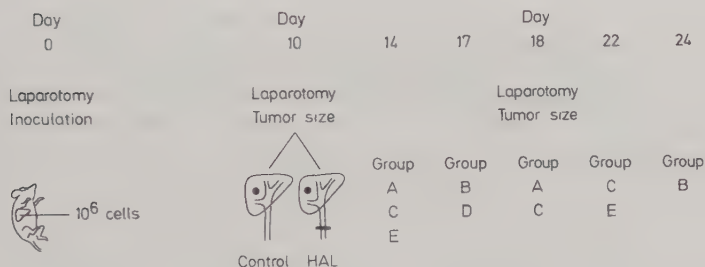


Fig 1. Design of the experiment.

In group A (18 Lister-hooded rats) a transplantable benzopyrene-induced sarcoma was inoculated. Group B (18 Lister-hooded rats) were inoculated with a transplantable hepatoma and group C (12 Lister-hooded rats) the same hepatoma 10 transplantations (approximately 100 days) later. Group D (12 Wistar rats) were inoculated with a N-methyl-N-nitrosoguanidine-induced adenocarcinoma in the colon and group E (12 Wistar rats) sixteen transplantations (approximately 160 days) later.

Ten days after inoculation the abdominal cavity was reopened under ether anesthesia and the liver tumor was measured with vernier calipers on the ventral surface of the central liver lobe at the greatest (a) and smallest (b) superficial diameters. Tumor volume was calculated according to the formula $V = a \times b^2/2$ (15).

In each group the animals were then randomized into subgroups, one hepatic artery ligation (HAL) group and one control group. There was no significant difference at that time between the tumor volumes within any one subgroup.

After isolation in the hepato-duodenal ligament the proper hepatic artery was ligated with an isolated 6.0 silk suture distal to the

gastro-duodenal artery (16). Control procedure consisted of tumor measurements as above.

At different intervals after hepatic artery ligation or control procedure, the liver tumor was measured under ether anesthesia by relaparotomies. At the last observation the animals were sacrificed, the shape of the tumors registered and the existence of macroscopical metastases investigated.

Statistical analyses were made on logarithmic values with Student's t-test and analyses of covariance.

RESULTS

At autopsy the tumors were in all cases solitary and ellipsoid in shape. No macroscopic metastases were found. No animals were found to be suffering from malnutrition during the experimental period. The weight of the animals before and after the experimental period showed no statistical differences in any animals in any group.

The cumulative mortality is listed in Table I.

Day for observation	Group A		Group B		Group C		Group D		Group E	
	Control n=9	HAL n=9	Control n=9	HAL n=9	Control n=6	HAL n=6	Control n=6	HAL n=6	Control n=6	HAL n=6
14	1	1			0	0			0	0
17			0	0			0	1		
18	1	3			2	0				
22					2	2			0	0
24			1	0						

Table 1. The cumulative mortality in different groups after hepatic artery ligation or control procedures performed on day 10.

The animals subjected to hepatic artery ligation in group A showed a higher mortality than the control group on day 18. No such difference was observed in any other group. The cause of death has not been analysed.

In group A there were no differences in geometric mean tumor volume on day 10 between the subgroups but there was a statistically significant larger tumor volume for the control animals compared with the ligated animals on day 14 and day 18 ($p < 0.002$) (Fig 2). Analyses of covariance on day 14 and day 18 adjusted for the tumor volume on day 10 showed a statistically significantly larger increase for the control animals compared with the ligated animals ($p < 0.001$) (Fig 2).

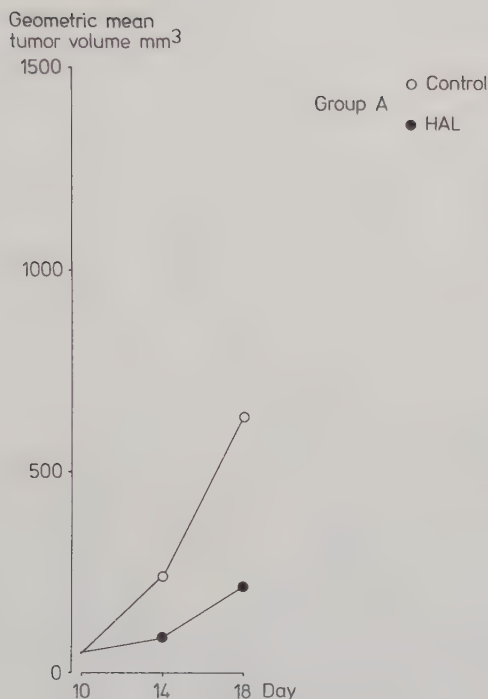


Fig 2. Geometric mean tumor volume for the animals inoculated with a benzepylene-induced sarcoma.

In group B there were no statistically significant differences in geometric mean tumor volume between the subgroups registered on day 10, 17 and 24 (Fig 3). There were no differences in the changes in the tumor volume between the control animals and the ligated animals during the observation period (Fig 3).

In group C a statistically significantly larger geometric mean tumor volume for the control animals compared with the ligated animals was observed on day 14 ($p < 0.001$), day 18 ($p < 0.001$) and on day 22 ($p < 0.0001$) (Fig 3). There was a statistically significant decrease in tumor volume for the ligated animals ($p < 0.002$) and an increase for the control animals ($p < 0.001$) between day 10 and 14. Between day 14 and 22 there was a statistically significantly larger increase in tumor volume in the control animals compared with that of the ligated animals ($p < 0.005$) (Fig 3).

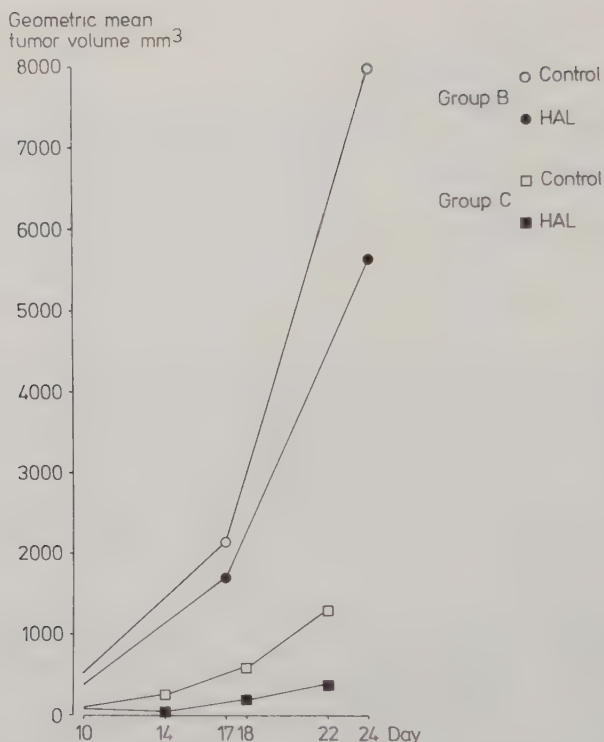


Fig 3. Geometric mean tumor volume for the animals inoculated with the hepatoma.

In group D there were no statistically significant differences in geometric mean tumor volume and in tumor growth rate between the control and the ligated animals (Fig 4).

In group E a statistically significantly larger geometric mean tumor volume for the control animals was registered on day 14 ($p < 0.0001$) and day 22 ($p < 0.0001$) compared with the ligated animals (Fig 4). The changes in tumor volume for the control animals from day 10 to day 14 showed a statistically significant increase ($p < 0.001$) and a statistically significant decrease for the ligated animals ($p < 0.001$). Analyses of covariance for the control animals showed a statistically significantly larger increase than for the ligated animals up to day 22 ($p < 0.001$) (Fig 4).

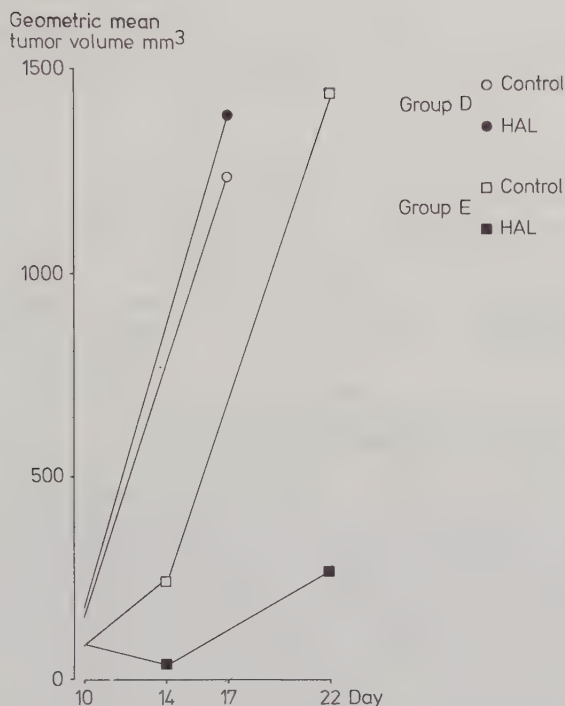


Fig 4. Geometric mean tumor volume for the animals inoculated with a N-methyl-N-nitrosoguanidine-induced adenocarcinoma.

DISCUSSION

Previous studies have shown that hepatic artery ligation (HAL) is the arterial inflow occlusion procedure which temporarily retards liver tumor growth (16). This effect was not found for regular liver dearterialization. Intraarterial Spongostan embolization also influences the growth of experimental liver tumors (14). Estimations of the effect of the HAL need frequent, repeated measurements of tumor size, which in experiments with liver tumors, involves laparotomy under anesthesia. This procedure influences the mortality rate and maybe even the results of the experiments as it is shown that surgical trauma stimulates an increase in liver tumor growth (17).

In the present study, in order to obtain frequent measurements of liver tumor volume, repeated laparotomies are undertaken every 4

days involving an increased mortality rate among the experimental animals. The mortality rate was negligible if the animals were subjected to a tumor measurement procedure every 7 days. A greater rate of mortality among the ligated animals compared with the control animals was only seen in the group that was also subjected to frequent laparotomies, implying that the surgical procedure of hepatic artery ligation per se was tolerated by the animals.

There were no fundamental differences in the effect of HAL on the different tumors. In all series maximum effect was registered on the 4th day. On that day a significant decrease in the tumor volume of the hepatoma and the adenocarcinoma but not in the sarcoma was seen. The explanation for this difference could be a faster re-vascularization of the sarcoma so the effect is exhausted already on the 4th day. The tumor blood flow for the hepatoma and the adenocarcinoma is of the same magnitude while it is much higher for the sarcoma (33).

Vascularized tumors are believed to be more sensitive to the interruption of the arterial blood supply but the findings in this study may indicate the contrary (20).

In this study it was also shown that tumors with high growth rate (groups B and D) are not so sensitive to interruption of arterial blood supply. This fast growth rate in these two groups, compared with the more moderate growth rate for the same tumor after several transplantations implies the necessary use of the same tumor generation in the same experimental procedure. This variation in growth characteristics of serial transplanted experimental tumors is well-known (6, 24).

The mechanism behind the temporary reduction in liver tumor growth after hepatic artery ligation is certainly complex and probably not exclusively explained by a reduced tumor blood flow. The influence of tumor blood flow and tumor cell metabolism of HAL has to be explored.

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Effects of Hepatic Artery Ligation and Intraarterial Embolization on Liver Tumor Growth—An Experimental Study in Rats

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and L. STIGSSON, MD

The effect of hepatic artery ligation and intraarterial embolization was investigated in rats with liver tumors. Only a temporary reduction in tumor growth was registered after both procedures as compared to controls. There was a somewhat more pronounced effect in the ligated animals than in the embolized ones. No clear cut differences in the extent of tumor necrosis could be found between the groups at the end of the experimental period.

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Key words: liver tumors, hepatic artery ligation, intraarterial embolization, angiography

INTRODUCTION

The outcome for any patient with liver cancer, primary or secondary, is very restricted. Median survival time is three months, and few patients survive more than one year [7]. Several modalities have been tried with the purpose of inducing tumor growth control, palliation, and longer survival—ie, liver resection, cytostatic infusion [2, 25].

Primary as well as secondary liver tumors receive their main blood supply from the hepatic artery [1, 9]. Hepatic artery ligation (HAL)—liver dearterialization—is a clinical experimental model based on the principle of inducing tumor necrosis by means of ischemia-anoxia [21]. In experiments using rats with liver tumors, HAL can give prolonged survival [20] and reduced speed of tumor growth [13]. HAL is now being performed with increasing frequency on patients to treat primary and secondary liver cancer but has not yet become clinically established [4], with the exception of patients with carcinoid syndrome from liver metastases [5].

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Another technique for inducing tumor necrosis based on the same principle as hepatic artery ligation is regional intraarterial embolization. Arterial embolization has been found to induce tumor necrosis in rats [18, 24]. This technique has been tried clinically in a few cases with liver cancer [15, 19].

The aim of the present study was to investigate if intraarterial Spongostan embolization causes an equivalent or more pronounced induction of tumor necrosis and restricts tumor growth more than hepatic artery ligation.

MATERIALS AND METHODS

Thirty male Wistar rats weighing 200–250 gm were used. They were fed *ad libitum* on a standard rat pellet diet and water. Twelve rats died during the experimental procedure leaving 18 animals to be studied. The experimental design is illustrated in Figure 1.

In ether anesthesia 0.1 ml of a standard cell suspension containing 1.0×10^6 cells of N-methyl-N-nitrosoquinidine induced transplantable adenocarcinoma per 0.1 ml [22] was injected into the central lobe of the liver through a midline incision. The technique for preparation and inoculation of the tumor cell suspension has already been described [12]. Hepatic artery ligation was performed in accordance with Carlsson and Hafström [13].

Angiography of the liver was carried out according to the technique described previously [16, 17] with the modification that all repeated angiographies were performed via inguinal incisions. The embolic material consisted of a suspension of Spongostan powder (99.3% gelatin, Ferrosan International, Denmark) in contrast medium at a concentration of 0.25 mg/ml which was injected into the celiac axis in a volume of 0.04–0.08 ml under fluoroscopic control [18].

On the tenth day after inoculation, the animals were anesthetized with ether, celiac angiography was performed, followed by a laparotomy through a midline incision. By means of angiography the size of the liver tumors and their vascularity was estimated. The magnification of the angiography was negligible (3%). At the laparotomy the tumors in the liver were measured in two diameters with vernier calipers. The product of the diameters was taken as tumor size.

The rats were divided into three groups with six rats in each: Group A (control group) was subjected to celiac angiography and laparotomy. Group B (HAL group) was subjected to angiography and laparotomy. During laparotomy hepatic artery ligation (HAL) was performed and a second angiography was carried out within 15 minutes to control the effectiveness of the procedure and to estimate the degree of collateral arterial circulation. Group C (embolization group) was, after angiography and laparotomy, subjected to transcatheter embolization in the celiac axis and a second celiac angiography was performed within 5–10 minutes to control the extent of embolization and to estimate the collateral circulation.

Four and 11 days later all the animals had a repeat angiography and laparotomy in order to measure the tumor size and estimate the degree of embolization, revascularization and collateralization of the liver, and the vascularity of the tumors. After the laparotomy on the 11th day the animals were sacrificed. Tumors were excised and fixed in 10% formaldehyde solution. Sections (4 μ m) from paraffin-embedded material were stained with hematoxylin-erythrosin. At microscopy special attention was paid to necrosis in tumors in relation to tumor size, as well as to necrosis in liver tissue.

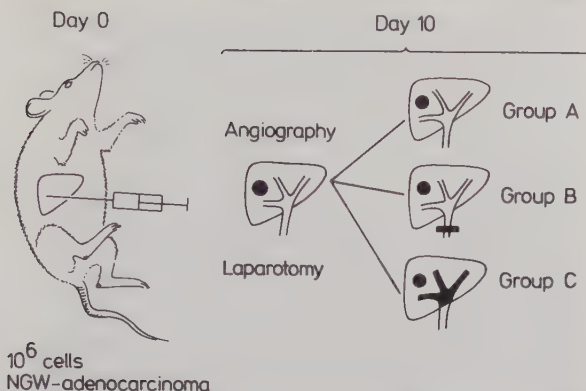


Fig. 1. Illustration of experimental model. Group A control series. Group B hepatic artery ligation (HAL) series. Group C embolization series.

The results are presented as mean and standard error of the mean (SEM). Comparisons between groups are made by means of students' *t* test for unpaired observations.

RESULTS

On the tenth day after tumor cell inoculation, the liver tumors measured by vernier calipers had the same size in all three groups. On the same day the first celiac angiography revealed neoplasms in 15/18 rats. The angiographic appearance of these tumors was the same as described previously [18, 24], ie, the center of the tumors appeared hypovascular with a hypervascularized rim. The size of the tumors measured by vernier calipers was in good agreement with the angiographic findings. In group B celiac angiography within 15 minutes after HAL revealed a complete interruption of hepatic arterial circulation via the hepatic artery. In all six rats collateral arterial circulation was demonstrated in the liver hilum with sparse contrast filling of the hepatic artery and its tributaries. No arterial circulation to tumors could be identified. The site of the hepatic artery ligation could be demonstrated in most animals. In five of the six animals subjected to embolization (group C) the angiography 5–10 minutes after the procedure showed a totally occluded hepatic artery. There was no peripheral circulation in the liver and no contrast filling of the tumors. No collaterals were identified in four animals. One animal had very few collaterals in the liver hilum. Another one had only a partial occlusion and faint contrast filling of the peripheral hepatic arterial branches.

Four days later the liver tumors had increased in size in all the animals in the control group (group A). The relative increase was 121% (Fig. 2). This increase was also verified in five animals by angiography (Fig. 3). In group B HAL induced a decrease in tumor size in all animals measured four days after ligation. This decrease was 42% (Fig. 2). In three animals the tumors could be demonstrated at the first angiography, and in two of these the size of the tumor had decreased (Fig. 4). In group

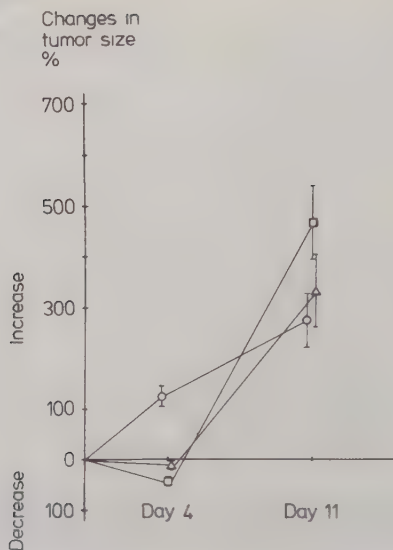


Fig. 2. Relative changes in tumor size after control procedures (○—○), after HAL (□—□), and after embolization (△—△).

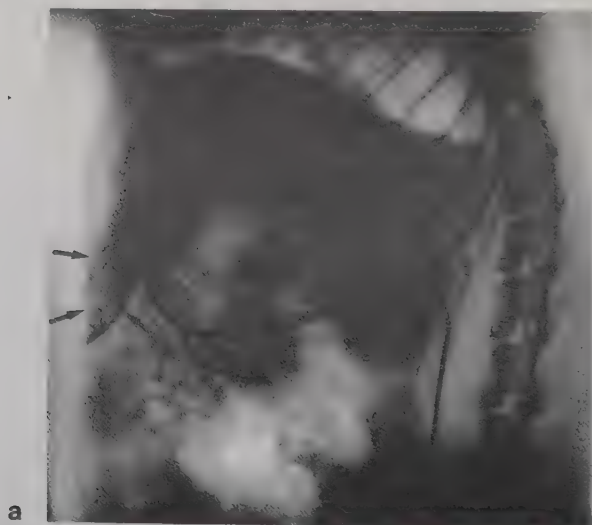


Fig. 3. a) Baseline celiac arteriogram in a rat from the control group. Moderately vascularized and well circumscribed tumor ventrally (arrows). b) Repeat angiography four days later. The tumor has increased in size but is less vascularized (arrows). c) Another seven days later tumor size is apparently unchanged as measured by angiography. However, the vascularization of the tumor has increased (arrows).

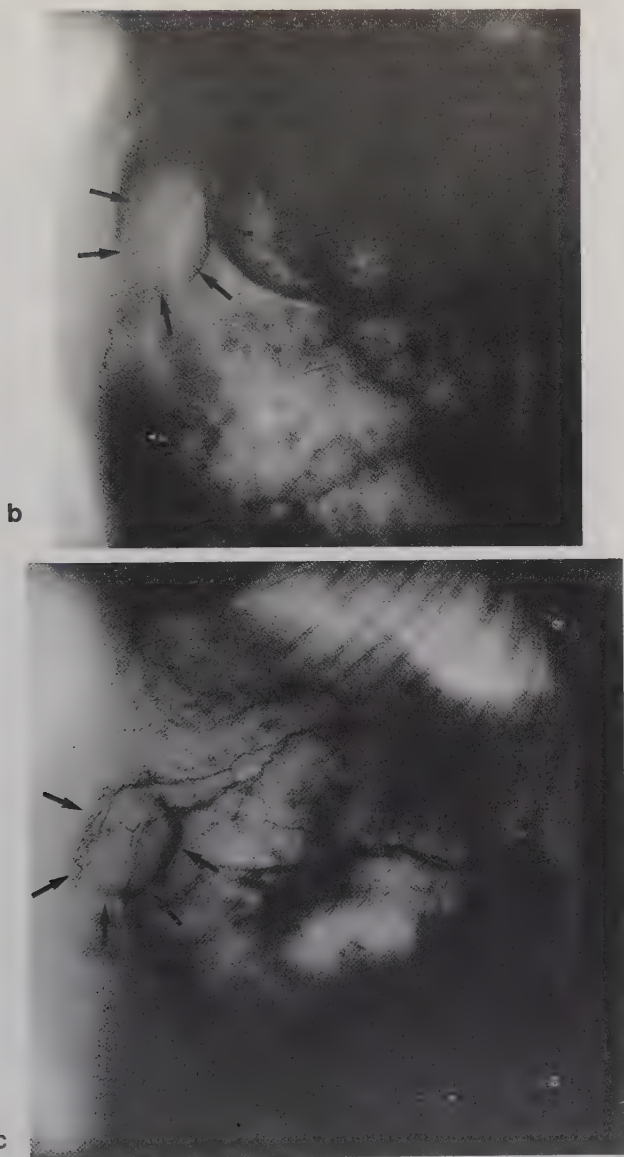


Figure 3.

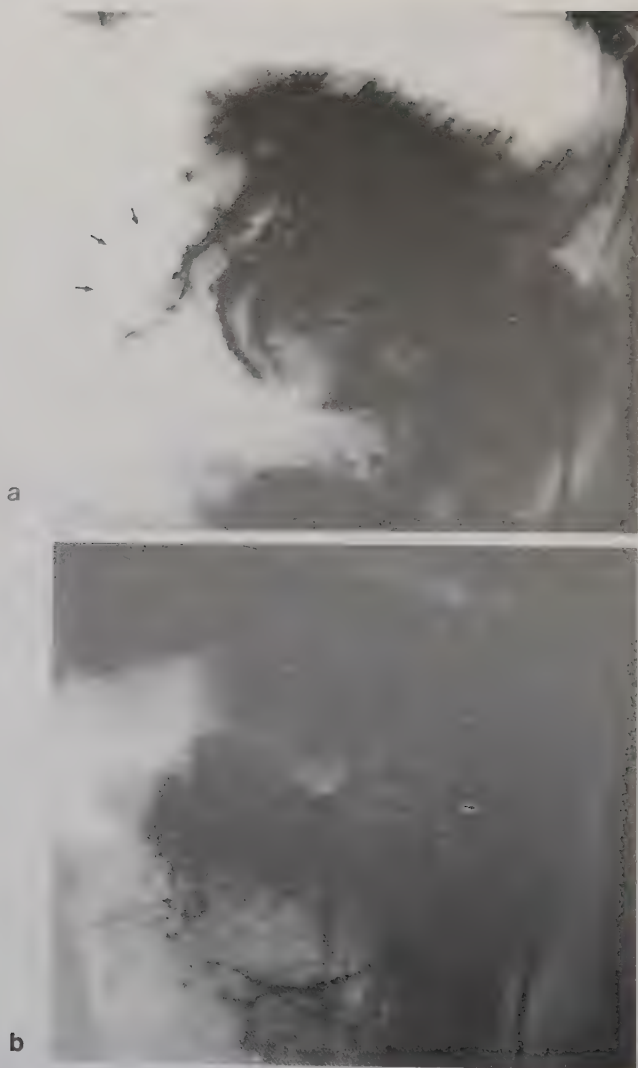


Fig. 4. a) Celiac angiography (lateral projection) immediately before ligation of the hepatic artery. Hypovascular tumor ventrally in the liver (arrows). b) Fifteen minutes after ligation. The hepatic artery (→) faintly opacified via collaterals. c) Four days later. Hepatic arterial circulation partly reconstituted. However, no contrast medium reaches avascular tumor. d) Further restitution of the hepatic arterial circulation 11 days after ligation. Faint contrast accumulation within the tumor.



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Figure 4.

C embolization was followed by a decrease in tumor size in five rats and an increase in one. The net effect was a decrease of 18% (Fig. 2). In this group two tumors were measurable at angiography and they had decreased in size (Fig. 5). The decreases in tumor size in the HAL group (group B) and embolization group (C) were statistically significant. These differed significantly from the increase in the control group (A) ($P < 0.001$). There was also a significant difference in the decrease between the HAL and embolization groups ($P < 0.05$) (Fig. 2). There was a statistically significant larger increase in the HAL group than in the control group between the fourth and 11th day ($P < 0.05$) (Fig. 2). No statistically significant differences were observed between group A and group C nor between group B and group C during this period (Fig. 2). The tumor size in the HAL group differed statistically from the control group on day 4 ($P < 0.05$) (Fig. 6) but did not differ from the embolization group. Neither was there any statistically significant difference between the control and the embolization group on that day. On the 11th day there were no differences in tumor size between the groups.

At histo-pathological examination the portal venule as well as the central veins were somewhat dilated in treated animals. One rat in the ligated group and two in the embolized group showed rather discrete but sometimes confluent midzonal and central necrosis in the liver parenchyma. The tumors were about the same size in all the groups. All the tumors in all three groups showed rather extensive central necrosis with a peripheral rim of preserved vital tumor tissue. No clear-cut differences between the groups concerning the extent of necrosis could be found.

DISCUSSION

According to earlier findings primary and secondary malignant hepatic tumors receive their main blood supply from the hepatic artery [1, 9]. It has been shown in man as well as in experimental animals that ligation or embolization of the hepatic artery results in liver tumor necrosis [4, 13, 18, 21, 24].

One possible explanation for the poor effects registered in humans after hepatic artery ligation is that the hepatic artery has not been completely ligated. In the present experimental model the procedures for inducing liver tumor necrosis, ligation, or embolization were controlled by the subsequent celiac angiography which clearly demonstrated that the hepatic artery really was occluded in all animals except for one embolized. Another explanation is that collaterals to the human liver develop very soon after hepatic artery ligation causing a rearterialization [8, 27]. The aim of embolization as opposed to ligation of the hepatic artery at its origin is to establish a more peripheral occlusion of the arterial flow which might result in the development of fewer collaterals [18]. There was in the present study a clear difference between the two groups with a much more pronounced tendency in ligated animals to develop collaterals and to establish early recirculation.

A disadvantage concerning embolization with Spongostan is the tendency for early revascularization. In this material there was a complete or partial recanalization in all animals of the vessels after four days, which is in good agreement with earlier findings [18, 24]. Repeat embolization could be used to obtain a more long-lasting effect. Other embolic materials that could be used for more permanent occlusion are, for instance, cyanoacrylate [14] or metallic coils [26].

There are several ways to evaluate the effects of the procedures. The best recognized way is to seek for variations in survival in the different groups. This requires,

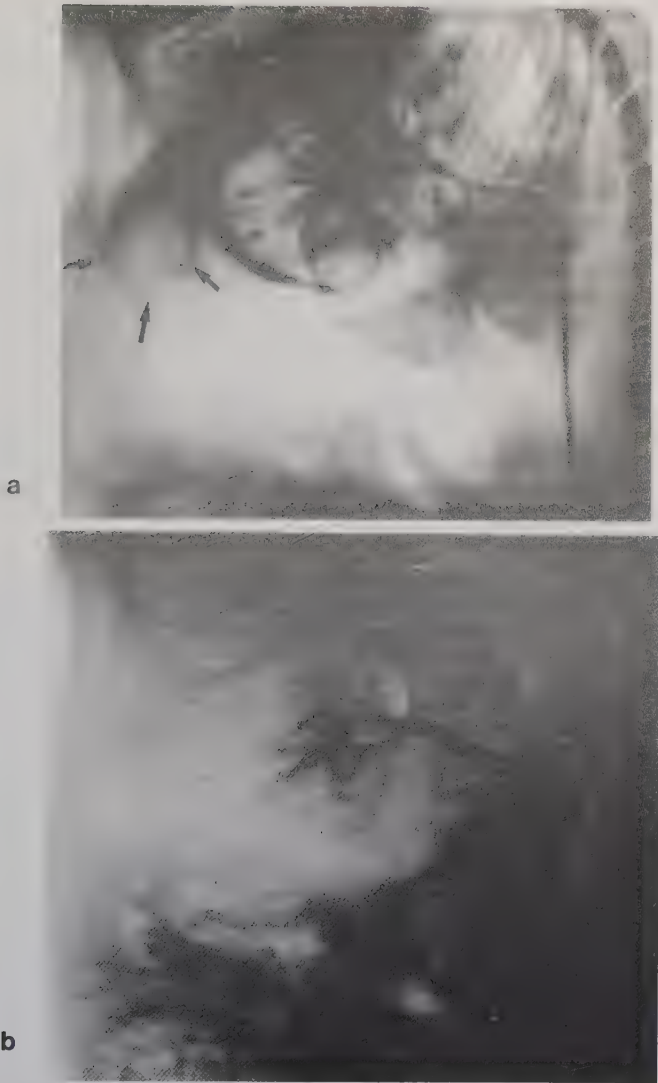


Fig. 5. a) Celiac angiography demonstrating hypovascular tumor in the central part of the liver (arrows). b) Ten minutes after embolization. Hepatic artery totally occluded in the periphery. No collaterals. c) Four days later. Hepatic arterial flow predominantly reconstituted. Decreased size of the tumor (arrows). d) Eleven days following embolization. Tumor has again increased in size and is somewhat more vascularized as compared to the initial arteriogram.



Figure 5.

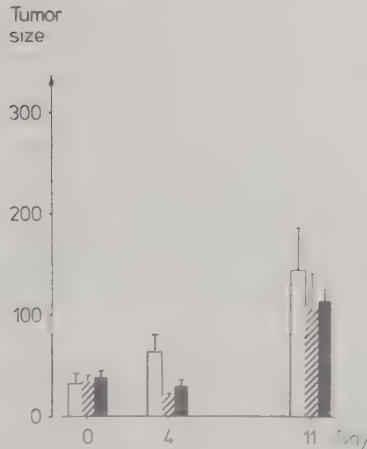


Fig. 6. Tumor size measured by vernier calipers in two dimensions. □, control; ▨, HAL; ■, embolization.

however, large series of experimental animals and even then minor differences between the procedures will not be revealed. In the present study the effects were registered by measuring the tumor size in two diameters by vernier calipers on the fourth and 11th day. This technique has been employed for a considerable time and the reproducibility is high [11, 24]. The angiography performed showed convincingly that the measuring could further support this technique for measuring the tumor [11].

The overall result after the two treatment techniques was a decrease in tumor size after four days as compared with controls. This verifies earlier results of the effect of HAL on rat liver tumors [13] which was found to be most pronounced on the fourth day. It is also in good agreement with earlier experimental results showing more pronounced tumor necrosis in rat liver 2-6 days after embolization compared to controls [18, 24].

On the 11th day no differences were found concerning tumor size either between the two treated groups or between treated groups and controls. The reduced speed in tumor growth up to the fourth day after HAL is followed by an increased tumor growth. The net effect on the 11th day was identical tumor size in all groups. This verifies observations from clinical as well as experimental studies that hepatic artery occlusion only institutes a temporary effect.

Besides recanalization and collateralization there are other possible reasons for this limited effect. One is that the portal tributaries widened after arterial embolization which could improve the nutrition to the tumors [18]. This could be borne out by the widening of the portal venules observed in this study. Based on experimental angiographic results after hepatic artery embolization, a compensatory increase of the portal flow has been proposed [10].

Another limiting factor to be borne in mind for both HAL and spongostan embolization is the risk of inducing focal necrosis in normal liver tissue which was observed in some animals.

The conclusion of this study is that neither ligation nor embolization are procedures that can be used selectively for treating tumors, at least in this experimental model. Almersjö et al [4] have also demonstrated clinically that hepatic dearterialization as a sole procedure is not enough to control tumor growth within the liver. The two circulatory obstructive procedures used must be combined with other kinds of treatment, eg, antitumor drugs, hyperthermic treatment, radiation, immunologic manipulation [3, 6, 25].

The small advantage of HAL to induce more prominent reduction in tumor growth up till the fourth day must be further evaluated in experiments with other tumors before any definite conclusion is drawn.

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THE EFFECT OF TOTAL BODY MICROWAVE HYPERTHERMIA AND HEPATIC ARTERY
LIGATION ON LIVER TUMORS - AN EXPERIMENTAL STUDY IN RATS

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Running title: Hyperthermia and hepatic artery ligation.

ABSTRACT

The effect of general microwave hyperthermia and hepatic artery ligation (HAL) was tested on Wistar rats with a transplanted N-methyl-N-nitrosoguanidine-induced adenocarcinoma in the liver. Total body hyperthermia (41.5°C for one hour, three times during 24 hours) was given on the same day as HAL, and one, two and three days after. HAL induced a slower tumor growth than untreated controls. No additive effect was registered when total body microwave hyperthermia was added to HAL. When hyperthermia was added two days after HAL there was a transient decrease in tumor volume as in the HAL series. Total body microwave hyperthermia added three days after HAL induced a faster tumor growth than after HAL alone. When hyperthermia was added the same day and one day after HAL there was a 50 % mortality.

INTRODUCTION

Most liver tumors, both primary and secondary, receive their main nutrition from the hepatic artery (1, 2). The hepatic artery ligation (HAL), probably causes nutritional deficiency, hypoxia and acidosis in a liver tumor which then may cause necrosis (3). Normal liver parenchyma will only suffer minor damage because the portal circulation is sufficient for nutrition and oxygenation (4).

In experimental animals - rats - the positive effect of HAL on liver tumor growth has been established (3, 5, 6).

Hepatic artery ligation causes a temporary reduction in liver tumor growth (7) which may be explained by the assumption that some areas of a liver tumor, essentially the peripheral ones, survive because they receive sufficient blood supply from the portal circulation (8). Another explanation is that the fast development of collateral arterial circulation to the liver seen after hepatic artery ligation will reestablish a tumor circulation (6, 9).

That hyperthermia has a greater destructive effect on malignant than on normal tissue has been verified in several in vitro and in vivo observations (10, 11). However, there are reports on the lack of effect (12, 13). Hypoxic cells are claimed to be more sensitive to heat than well-oxygenated ones (14, 15) but no observed effect has also been reported (16). Interruption of both the artery and vein from a tumor dramatically increases the response of malignant tumor cells to hyperthermia (17). Repeated treatment with hyperthermia is more effective than single treatment (18).

Hyperthermia can be administered locally or generally by several techniques, i.e. radiofrequency, microwaves etc (11, 12). To direct local hyperthermia to a liver tumor a laparotomy is probably necessary and this may then aggravate problems concerning the administration of repeated exposures to hyperthermia.

Total body hyperthermia, general hyperthermia, has been tested in clinical situations with some evidence of a positive effect on the tumor (11, 19, 20).

The maximum tolerated exposure of total body microwave hyperthermia for rats has been found to be 41.5°C for three one-hourly sessions over 24 hours. After administration of such repeated hyperthermia growth of experimental liver cancer was reduced for four days (21).

By combining HAL with total body hyperthermia it can be assumed that the liver tumor cells will be hypoxic, acidotic and probably more sensitive to hyperthermia. Hyperthermia added to hepatic artery ligation may therefore cause a more pronounced effect on tumor growth than the procedure of hepatic artery ligation alone.

The present study was designed to investigate if HAL could add to the effect of total body microwave hyperthermia for the restriction of the growth of experimental liver cancer. It was also of importance to see how the experimental animals tolerated this combined procedure.

MATERIAL AND METHODS

Thirty-six inbred Wistar rats of both sexes with an age of 5-6 months, and a weight of 200-250 g were maintained on a standard pellet diet and water ad libitum.

A cell suspension containing 0.5×10^6 viable tumor cells per 0.1 ml of a transplantable adenocarcinoma of the colon induced by N-methyl-N-nitrosoguanidine was used. In ether anesthesia through a midline incision, 0.1 ml of the cell suspension was inoculated into the central lobe of the liver (day -5) (Fig 1) (22). Five days later (day 0) (Fig 1) all animals were anesthetized and through a laparotomy the tumors were measured at the greatest (a) and smallest (b) superficial diameters on the ventral side of the liver lobe with vernier calipers. Tumor volume was calculated with the formula $V = a \times b^2 / 2$ (22).

The animals were then randomized into six groups with six animals in each (Fig 1): group A control, group B ligation of the hepatic artery distal to the gastroduodenal artery (HAL), group C HAL and total body microwave hyperthermia on the same day, group D hyperthermia on the following day, group E on the second day and group F on the third day after HAL (Fig 1).

The hyperthermia was induced by microwaves generated from a Siemens magnetron (Radiotherm 305 at 2.45 GHz max and an output of 200W). The exposure was below 4.000 W/m^2 . The temperature probe placed in the rectum was connected to a computerized system (INTEL 8085 microprocessor with a 16 kbyte memory). The microwave generator was set through a series of relays at a temperature of 41.5°C for one hour three times during 24 hours, i.e. every 8 hours. To avoid microwave-induced interference the generator was turned off for 4 s before reading the temperature. The rectal temperature was recorded every 30 s during the 24 hours. The microwave generator induced hyperthermia with a precision of $\pm 0.1^\circ\text{C}$ of the predetermined temperature (23). During the 24 hours of microwave hyperthermia the animals were kept isolated with water and food in a special plexiglass cage. The tail and temperature probes were fixed through a hole in the cage. This cage was then placed in a bigger plexiglass cage with a constant temperature of 25°C . After the 24-hour period the animals were placed in their regular animal cages. No anesthesia was given during the hyperthermia.

Five days after the HAL (day 5) (Fig 1) the animals were laparotomized and the size of the tumor was measured in the same way as before. This procedure was repeated two days later (day 7) (Fig 1) and then the animals were sacrificed. Mortality during the seven days was registered. Statistical analyses were made on logarithmic values with Student's t-test and analyses of covariance.

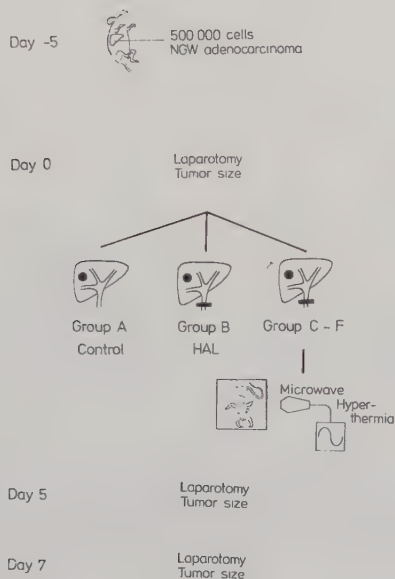


Fig 1. Experimental design.

RESULTS

No mortality was registered in the control group (group A) or the group subjected only to hepatic artery ligation (group B). In the group subjected to hyperthermia the same day (group C) and the day after HAL (group D) three out of six animals died before day 5. In the two other groups (groups E and F) one of six animals died before day 5. No mortality was registered in any group after day 5. In all cases the animals died during the period of hyperthermia.

In all animals at autopsy the tumors were solitary and ellipsoid in shape in all the groups. No macroscopic metastases were found. Because of the small number of surviving rats in groups C and D on

day 5 and 7 these groups are not subjected to statistical analyses. The weight of the animals did not change statistically in any animals in any group during the experimental period compared with the weight at the beginning of the experiment.

On day 0 there were no statistically significant differences in geometric tumor volume between the groups (Fig 2). Five and seven days later there was a statistically significantly larger tumor volume in group A and group F compared with groups B and E ($p < 0.0001$). On day 7 the tumor volume in group A was statistically significantly larger compared with group F ($p < 0.005$) but the tumor volume in group F was statistically significantly larger compared with groups B and E ($p < 0.0001$).

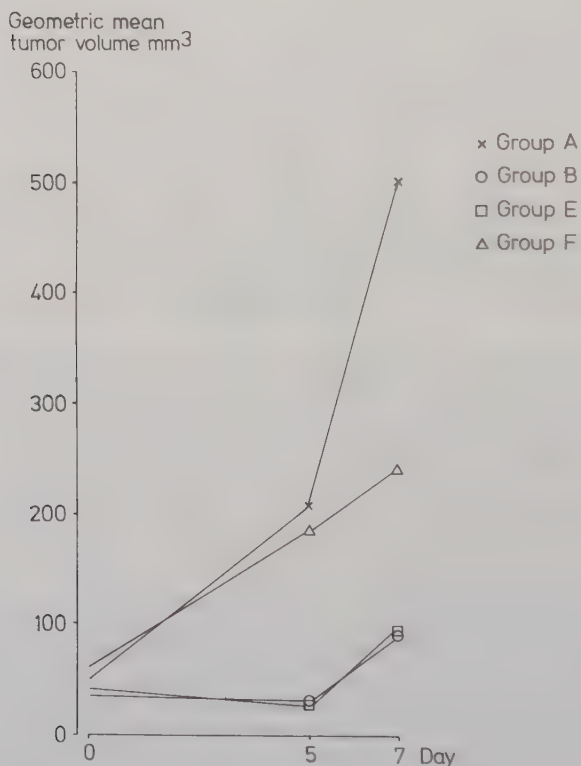


Fig 2. Geometric mean tumor volume for the four analysed groups during the experimental period.

There was a statistically significant decrease in tumor volume from day 0 to day 5 in group E ($p < 0.04$) while in groups A ($p < 0.001$) and F ($p < 0.04$) there was an increase (Fig 2).

Analyses of covariance on day 5 showed a statistically significantly larger increase in groups A and F than in groups B and E ($p < 0.0001$) (Fig 2). On day 7 the control animals (group A) showed a statistically significantly larger increase compared with group F ($p < 0.005$) and groups B and E ($p < 0.0001$) and group F showed a larger increase compared with groups B and E ($p < 0.008$).

DISCUSSION

The outcome for any patient with liver cancer is so restricted that any rational way of attacking this disease is warranted (24). Liver dearterialization (HAL) has been used for this purpose for more than 10 years and no evidence of its value as a single procedure in patients has been established (25).

Hepatic artery ligation probably produces some hypoxic and anoxic tumor cells because of the dominant arterial supply of the tumors (1, 2). These hypoxic tumor cells will probably be more sensitive to the addition of hyperthermia than normal liver cells which are sufficiently oxygenated by the portal circulation. The temporarily diminished blood flow through the tumor may create a hot spot in some areas of the tumor under the concomitant hyperthermia. Normal liver parenchyma should maintain a normal temperature due to the cooling by the portal circulation.

The assumed additive effect of hyperthermia to hepatic artery ligation cannot be verified in this study. Hyperthermia added two days after hepatic artery ligation gave the same effect as the hepatic artery ligation alone but total body microwave hyperthermia added three days after HAL induced an increased tumor growth when compared with only HAL.

The reason for this stimulation of tumor growth is difficult to explain. Combination with hyperthermia on the third day after hepatic artery ligation means that hyperthermia is added to tumors which probably were larger than on the second day. Another explanation is that the effect measured in tumor volume may not truly reflect the tumor growth because an oedema may be present two days after hyperthermia. This postulated oedema may disappear very soon and is therefore not manifest in the other groups on the fifth day when all animals were examined.

A study with more frequent examination of tumor volume and tumor histology after the combination therapy with hepatic artery ligation and total body microwave hyperthermia is required for further analysis in this field.

When total body microwave hyperthermia was applied the same day and the day after HAL, the mortality was higher (50 %). This mortality was registered on the day the animals were submitted to hyperthermia. The reason for this high mortality has not been analysed in the present study. Possible explanations are disturbed homeostasis and/or liver failure.

The optimal time for administration of hyperthermia should reasonably be before development of collaterals. Development of arterial collateral circulation to the liver is verified by an angiographic technique as soon as 10-15 min after HAL and the tumor circulation was re-established four days later (6).

The results of this study indicate that total body microwave hyperthermia combined with HAL does not cause a synergistic or additive effect on liver tumor growth. Moreover, hyperthermia added to HAL is accompanied by high mortality.

These findings may motivate a test of the effect of local hyperthermia and concomitant hepatic artery occlusion.

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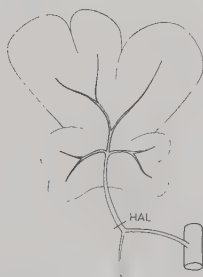
HEPATIC TUMOR GROWTH

Influence of hepatic artery occlusion.

An experimental study in rats.

by

Göran Carlsson



Lund 1982

To Gunilla and Joachim

This thesis is based on the following papers:

- I. SOLITARY EXPERIMENTAL LIVER TUMORS IN RATS. Carlsson G, Gullberg B and Hafström Lo.
Submitted for publication.
- II. VASCULARIZATION AND TUMOR VOLUME ESTIMATIONS OF SOLITARY LIVER TUMORS IN RATS. Carlsson G, Ekelund L, Stigsson L and Hafström Lo.
Submitted for publication.
- III. INTERRUPTION OF HEPATIC ARTERIAL SUPPLY IN RATS WITH LIVER TUMORS. Carlsson G and Hafström Lo.
Submitted for publication.
- IV. INFLUENCE OF HEPATIC ARTERY LIGATION ON LIVER TUMOR GROWTH IN RATS. Carlsson G and Hafström Lo.
Submitted for publication.
- V. EFFECTS OF HEPATIC ARTERY LIGATION AND INTRAARTERIAL EMBOLIZATION ON LIVER TUMOR GROWTH - AN EXPERIMENTAL STUDY IN RATS. Carlsson G, Ekelund L, Hafström Lo, Jonsson N and Stigsson L. J Surg Oncol 17:249-261, 1981.
- VI. THE EFFECT OF TOTAL BODY MICROWAVE HYPERTHERMIA AND HEPATIC ARTERY LIGATION ON LIVER TUMORS - AN EXPERIMENTAL STUDY IN RATS. Carlsson G, Hafström Lo, Hugander A, Jönsson P-E, Bolmsjö M and Persson B.
Submitted for publication.

In the text these papers will be referred to by their Roman numerals.

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INTRODUCTION

The normal liver

The liver plays an important part in the metabolism of carbohydrates, protein and fat, in detoxication, the formation and secretion of bile, the synthesis of vital proteins, and as a storage organ (1).

Anatomically the liver is a segmental organ where each segment receives branches from the hepatic artery and the portal vein. These segments emit veins and bile channels which join the hepatic veins and the bile ducts, respectively. In humans the liver is divided into eight separate segments while the rat liver contains five (2, 3).

The liver has a dual blood supply from the hepatic artery and portal vein. Arterial blood supply normally originates from the coeliac axis, but there is a great variation in the anatomy of the arteries that run to the liver (4). Through the hepatic artery the human liver receives 35 % of its blood supply and 65 % from the portal vein. The oxygen supply to the liver is equally shared by the portal vein and the hepatic artery (5). The proportion between the hepatic artery and portal vein regarding blood flow and oxygen content in normal rat livers has not been found in the literature.

Liver tumors

In a malignant tumor the cells have escaped from restrictions and got caught up in a continuous dynamic, frequently changing and complex series of events. Tumor growth in an organ is dependent on the organ and the number of tumor cells.

In the earlier phase of growth neoplastic cells exchange nutrition and catabolites with the surrounding interstitial tissue by simple diffusion. This phase is referred to as the avascular phase during which tumor growth is arrested. When formation of stroma and vessels is established the neoplastic tissue multiplies to constitute a solid tumor (6). Proliferation of capillaries into the tumor and synthesis of collagen are induced and stimulated by the neoplastic cells (7, 8).

Tumor growth in the liver is frequent in man. Next to lymph nodes, the liver is the most common organ for metastatic malignant growth. Primary liver cancer is rather unusual and often associated with liver cirrhosis (9, 10). Metastases from malignancy of the digestive tract are the most often occurring secondary tumor in the liver (11). Patients with untreated primary or secondary liver malignancy have a very limited survival. Median survival is six months or less (11, 12, 13). Longer survival has been reported but only a few patients survive more than two years (14, 15).

In cancer research, human tumors transplanted to experimental animals (16, 17) and tumors arising spontaneously in animals can be used (18, 19). However, the usual method is to employ carcinogenic substances to induce tumor growth (20, 21, 22). Although spontaneously or chemically induced experimental tumors bear the closest resemblance to the human situation they have their disadvantages. For instance, the thereby achieved tumor growth in the liver is scattered, making accurate repeated tumor size measurements in living animals difficult. Another disadvantage with chemically induced tumors is that they are often of varying histological types (20, 22). Chemically induced experimental tumors can be used for transplantation in an inbred strain of experimental animals. This can be performed either in the form of a fragment of the tumor (18, 23, 24) or a suspension of tumor cells (25, 26). The amount of neoplastic tissue transplanted can be estimated from the number of viable tumor cells in the cell suspension or from the volume or weight of the tumor fragment (27, 28). The cell suspension technique and the fragment transplanting technique induce one solitary tumor which enables the estimation of tumor size in a living animal. Inoculation of tumor cells in order to induce liver tumors in experimental animals can be performed either by injection into the portal vein or the hepatic artery or straight into the parenchyma (25, 29, 30, 31, 32). The benefit of portal injection is that it mimics the clinical situation with dissemination from gastrointestinal carcinoma. The disadvantage of portal injection is that it results in scattered tumor growth in the liver (30).

The amount, viability and age of the transplanted neoplastic tissue influence the tumor take and growth. Repeated transplantations lead to changes in growth characteristics and histological type (33, 34). Earlier tumor take and larger tumor volume can be achieved in the liver of the rat via portal injection of tumor cells, by inoculation of a great number of tumor cells (30), by exposure of the liver to direct trauma or the experimental animal for surgical trauma as repeated laparotomies (35, 36, 37), by increasing or decreasing the liver blood flow (38, 39), by maintaining the rats on a high protein diet (40), by blocking or stimulating RES (41, 42) and by administering high molecular solutions (43). Administration of anticoagulants has shown to lead to a low frequency of tumor take and small liver tumor volume (44).

The vast majority of solid tumors in man and animals grow in a great number of tissues in the form of three-dimensional aggregates such as spheroids or ellipsoids (45).

Accurate estimations of the tumor size are in both clinical and experimental practice necessary in order to evaluate the effect of different therapeutic procedures. One-dimensional measurements of solid tumors give a rough estimation of tumor size (46) and measurements in two dimensions increases the reliability (19, 47). The product of measurements in two dimensions of solid tumors growing subcutaneously well express the tumor size. Repeated measurements

in two dimensions can thus be used to follow tumor growth of subcutaneous solid tumors, provided that the growth characteristics and the shape of the tumor do not change during the experimental period (19). Repeated measurements of tumor volume form a reliable parameter for the expression of tumor growth rate (48). Tumor volume can be calculated according to different formulas for measurements in two (49, 50, 51) and three dimensions (18, 23). Superficially growing tumors can be measured in three dimensions with good reliability but tumors in deep parenchymal organs, e.g. the liver, are difficult to measure in all three dimensions. The need for repeated measurements of an unremoved liver tumor creates a demand for an accurate formula to calculate the volume. It is therefore necessary to test different formulas for tumor volume calculation of an unremoved liver tumor in an experimental model.

The vascular pattern and blood flow of liver tumors

In humans injection methods of livers with secondary tumors have demonstrated that they receive their vessels from the hepatic artery (52, 53). In 1951 Bierman confirmed this observation by arteriographic investigations (54). The arterial vascularity of primary and secondary human liver tumors varies according to histological type (55, 56). Each type in itself shows great variation in vascularity. Usually the liver tumors were more vascularized than the surrounding liver (55, 56). However, the influence of tumor size on vascularity was not analysed.

In experimental animals the vascular pattern of liver tumors also reveals vessels emanating predominantly from the hepatic artery. This has been demonstrated by injection methods, arteriography and portography (20, 29, 57, 58). This observation remains true independent of whether the experimental liver tumors were induced by hepatocarcinogenic substances (20, 29), transplanted as a tumor fragment (57) or transplanted as a cell suspension in the portal vein, the hepatic artery or direct into the liver parenchyma (29, 58).

The vascular pattern varies, however, according to the tumor type and tumor size. Nilsson and Zettergren (20) demonstrated by arteriography that well differentiated experimental hepatocellular carcinoma were more vascularized than poorly differentiated. The contrary was found for cholangiocarcinoma (20). Portography of the same tumor types revealed portal vessels running into a well differentiated hepatocellular carcinoma. In the poorly differentiated tumors the portal vessels were chiefly localized in the peripheral parts. In the cholangiocarcinoma no portal vessels were observed anywhere (20). These findings confirmed the results found by Honjo and Matsumura, who studied the same types of experimental liver tumors by injection of dye solutions (59).

The relation between tumor size and vascular pattern has been

investigated by Ackerman with injection of silicone rubber (57). Tiny tumors (diameters 1-2 mm) were encircled by vessels from either arteries or veins or both. Larger tumors (diameter 3-7 mm) were supplied by arterial vessels while massive tumors (diameter 7-33 mm) had a vascular pattern predominantly arterial or predominantly portal or a combination of the two. This study was performed with solitary Walker-256 carcinosarcoma implanted in the liver. With the same technique portal vessels have been demonstrated in the peripheral parts of the tumor (24, 60).

With hepatic angiography Kido investigated the vascularity of Walker-256 carcinosarcoma inoculated intrahepatically as a cell suspension (61). When the tumor volume increased the central part became more avascular. However, the used angiographic technique made repeated investigations impossible in the same animal. Honjo and Matsumura studied the same type of liver tumor with injection of dye solutions and found portal vessels sporadically within the tumors (59).

The relative blood flow measured with RISA and microsphere techniques of the same tumor type was also studied by Ackerman (24, 28). Small liver tumors (less than 0.3 g) receive their blood flow from both the hepatic artery and portal vein but increased tumor size was accompanied by increased blood supply from the hepatic artery. With the same techniques Sugahara et al have found contradictory results in tumors induced by hepatocarcinogenic substances (22). They found these liver tumors nourished predominantly from the hepatic artery but with increasing tumor size the arterial blood flow became less predominant. The vascular bed and relative arterial and portal tumor blood flow have been found to decrease with increasing tumor volume (22). In liver tumors larger than 1.5 g the periphery is more perfused with arterial blood than the central part (25).

The proportion between tumor and liver blood flow seems to vary considerably in different investigations. With RISA and microsphere techniques the tumors in rats have been found to be more perfused with blood than the normal liver parenchyma (22, 24, 28). There are also reports of an equal blood flow to tumor and liver parenchyma and even less blood flow to the tumor than to the liver parenchyma has been found (25, 62). In humans ¹³³Xenon injected into the liver metastases have revealed that the metastases are less perfused than the normal liver parenchyma (63, 64).

The vascular pattern of liver tumors induced by inoculation of tumor cells intraparenchymally has been based on investigations in only two species (rabbit and mouse) (29, 58). The relation between tumor size and variation in vascular pattern has been based mainly on postmortem investigations (24, 57, 61). It seems therefore reasonable to study the variation in vascular pattern of unremoved liver tumors induced by intrahepatically inoculated tumor cells in rats.

Therapeutic attempts

In order to improve the survival for patients with liver malignancy different therapeutic procedures are used.

1. Liver resection

At present liver resection is the only method to cure liver malignancy (65). Ideal conditions for liver resection are a resectable localized tumor that shows no evidence of extrahepatic tumor growth. Primary or secondary carcinoma of the liver is, however, in most cases scattered within the liver and this influences the frequency of curative operation (66). Figures up to 81 % in three-year survival have been reported for patients subjected to curative resection. This contrasts with the three-year survival of 18 % for patients with palliative resection (66).

Total hepatectomy and transplantation of the liver has been tried but so far this procedure is an exclusive method for treatment of liver malignancy (67).

2. Hepatic arterial interruption

Historically, interruption of the hepatic arterial blood flow was claimed to be a procedure which in most cases was lethal for the patients (68). The anatomical investigations in humans which claimed that the liver arteries are end arteries was believed to be the reason for the deleterious effect of hepatic arterial interruption (52). Already in 1920 there was a report of contradictory findings by Martens who by arteriography demonstrated the existence of intrahepatic arterial anastomoses (69). This finding has been further confirmed with the same technique in recent studies (70, 71). Even with improved postoperative care accidental interruption of the arterial blood supply to the liver places the patient in a severe situation (72). Interruption of the proximal hepatic artery is more tolerable than distal interruption (68, 73, 74). In experimental animals administration of antibiotics, hyperbaric oxygen and shunts between the portal and the inferior caval veins was found to decrease mortality after interruption of the hepatic arterial blood supply (75, 76, 77).

Portal blood flow is capable of protecting the liver parenchyma from severe damage when the arterial blood supply is interrupted because of the increased employment of existent oxygen in the portal blood (5). The arterial collaterals to the liver supply further support after HAL.

In humans and experimental animals occlusion of the portal vein in normal livers has been shown by electromagnetic flow probes to be accompanied by an increase in the hepatic arterial blood flow (78, 79, 80). The reports of the effect of interruption of hepatic arterial blood flow on portal blood flow are inconsistent.

Increased portal blood flow has been reported in isolated perfused canine livers (81). In situ experiments in humans and experimental animals have shown unchanged portal blood flow during a few minutes of occlusion of the hepatic artery (78, 79, 80). Permanent or temporary occlusion of the hepatic artery for up to one hour in experimental animals is accompanied by a decrease in the portal blood flow as measured with 133-Xenon, thermodilution or electromagnetic flow probe techniques, but portal blood flow is restored within minutes after the temporary occlusion is released (82, 83, 84) or within one day after permanent HAL (85).

The total liver blood flow in normal livers in humans and in experimental animals measured with 133-Xenon has been found to decrease after up to one hour of temporary occlusion of the hepatic artery but recovers within minutes after the release of the arterial blood flow (84, 86). Observations of an unchanged total liver blood flow after permanent interruption of the hepatic arterial blood flow have also been reported but the blood flow was measured first 15 days after permanent interruption of the hepatic arterial blood flow (87).

Ligation of the portal vein in experimental animals with liver tumors results in a shunting of arterial blood from the tumors to the liver parenchyma (88). In humans and experimental animals with liver tumors an interruption of the hepatic artery leads to a more reduced blood flow in liver metastases than in normal liver tissue (22, 63, 64, 88, 89). Recovery to preoperative values takes place within 4-5 days or up to 4-6 weeks after permanent occlusion (63, 88) and immediately after release of temporary occlusion (64).

Injection of silicone rubber in experimental animals with liver tumors have shown portal vessels leading to the periphery of the tumors after HAL (24, 60). This finding is supported by measurements with 133-Xenon showing increased portal blood flow to the periphery of colorectal metastases in humans after HAL (64). This is, however, based on measurements in patients previously subjected to intravascular 5-FU infusion through the hepatic artery or portal vein.

In the periphery of liver tumors in humans and in animals vital tumor tissue has been observed after HAL (90, 91).

The above mentioned observations of variation in blood flow to normal liver and in livers occupied by tumors before and after interruption of arterial blood flow relate to a great deal to the method used for measurements of hepatic blood flow (92).

An arterial revascularization of the liver after permanent hepatic artery ligation is capable of restoring the hepatic arterial circulation. This revascularization is caused by enlargement of pre-existing arterial collaterals to the liver. This is demonstrated by angiography to occur minutes and hours and up to three to five days

after hepatic artery occlusion both in humans and experimental animals (71, 93, 94, 95). This fast revascularization of the liver makes complete and permanent interruption of the hepatic arterial blood supply more or less impossible.

Decrease in tumor size, microscopic tumor necrosis without gross changes in normal liver parenchyma are observed from five days up to six months after interruption of the hepatic arterial blood supply in humans and experimental animals (22, 90, 91, 96, 97, 98, 99, 100). The degree of tumor necrosis is related to when the microscopic examination is performed. Necrosis is more pronounced five days than 21 days after HAL (98). The duration of the influence on liver tumor growth after HAL has not been analysed. Neither when the most pronounced effect occurs.

The reduced arterial blood flow to the liver after HAL leads to a shortage of oxygen and substrate. This can be deleterious for the liver cells with their high metabolic rate (101). Scattered small liver infarctions have been observed in normal human liver tissue three days after HAL (97). Permanent interruption of the arterial blood supply to the liver in humans and experimental animals induced a transient increase of GOT, GPT, LDH, OCT in serum with a maximum value after one to three days and recovery after one week (102, 103, 104, 105). Transient decrease in mitochondrial function, liver glycogen content, amino acid incorporation into liver proteins, ribosomal function, microsomal enzymes and coagulation factors was reported after temporary or permanent occlusion of the hepatic artery in humans and experimental animals. Recovery to normal function was seen within hours after the release of the temporary occlusion of the hepatic artery or within one week after permanent occlusion (22, 105, 106, 107, 108, 109, 110, 111).

In experimental animals without liver neoplasia a transient decrease in hepatic oxygen tension, hepatic oxygen uptake and hepatic oxygen consumption has been observed after temporary occlusion of the hepatic artery (83, 84, 112, 113). Recovery takes place within one hour after reopening the artery (83, 84) or after one day following permanent occlusion (113). Contradictory findings have been reported in humans to the effect that an unchanged hepatic oxygen consumption has been measured after temporary occlusion of the hepatic artery for 24 minutes (5). Unchanged hepatic oxygen tension is also observed after arterio-portal shunts and 60 % oxygen breathing following HAL (112, 113). The oxygen extraction of the liver has been reported to increase during one hour of temporary occlusion and up to 30 minutes after reopening of the hepatic artery (84).

A major etiological factor for cell necrosis in neoplastic tissue following interruption of the arterial blood supply has been assigned to lack of oxygen. Hypoxia is known to reduce tumor growth (114) but the lack of oxygen is not yet proven to be the only etiological factor behind necrosis following hepatic artery liga-

tion. Lack of nutritional factors and accumulation of catabolites may also be of importance. In order to study metabolic and oxygen variations in normal liver tissue and variation in the growth rate of neoplastic tissue in the liver, a standardized and reproducible method for inducing liver tumors and reducing the arterial blood supply to the liver is necessary.

Markowitz' speculation in 1952 that hepatic artery ligation could be useful in the treatment of secondary liver malignancy was based on the finding that the blood supply to hepatic metastases was predominantly arterial (115). Interruption of the hepatic arterial blood supply, although accidentally, in patients with liver malignancy had, however, been reported earlier. (116). Hepatic artery ligation was introduced in the clinic by Nilsson in 1966 as a method to control metastatic growth in the liver (97).

The arterial blood supply to the liver occupied by neoplastic growth can be interrupted either by ligation of the hepatic artery or by the more extensive procedure of liver dearterialization including separation of the liver from all surrounding attachment except the hepatic veins, portal vein and common bile duct (97, 99, 117, 118, 119, 120). At present it is not possible to say which of these procedures is preferable for the interruption of the hepatic arterial blood supply. More information may be achieved from further experimental studies. In experimental animals with liver neoplasia, hepatic artery ligation has been reported to prolong the median survival compared with untreated animals (90). No statistical analysis was performed in this study. In the clinic, the best positive effect of hepatic artery interruption is observed in patients with carcinoid metastases in the liver in whom reduced symptoms and decreased urine release of 5-HIAA are observed (117, 119, 120). In patients with primary or metastatic liver cancer hepatic arterial interruption has not yet been proved to be valuable as a single procedure (118, 121). In a randomized clinical study in patients with colorectal metastases HAL, though in combination with a rather small dose of intraarterial 5-FU, gave an identical survival as untreated controls (122). However, this finding was based on a restricted number of patients.

Temporary occlusion of the hepatic artery by laparotomy or by an intravascular balloon catheter may have advantages over permanent occlusion but no reports have yet emerged to this effect (121, 123, 124).

Further knowledge in this field has to be gained in both experimental animal research and prospective randomized clinical studies. Basic research on the effect of therapeutic procedures on liver tumor growth has to be performed in animals. A standardized experimental model for hepatic artery occlusion would probably be profitable.

3. Intravascular cytostatic agent infusion to the liver

Infusion of cytostatic agents by percutaneous catheterization of the hepatic artery via the brachial artery or by peroperatively introduced catheters via the gastroduodenal artery to the hepatic artery is employed in order to attain a local high-dose of the drug with a minimum of side-effects (125, 126, 127). The most commonly used drug is 5-fluorouracil. This technique has probably improved the survival and quality of life for the patients who responded to the treatment but no randomized study with an untreated control group has so far been reported (128, 129, 130). When intraarterial 5-FU infusion was tested versus systemic 5-FU in a randomized manner by Grage et al (131) no difference in response rate, duration of remission or survival was found. It should be emphasized, however, that the intraarterial infusion was given for one restricted period of 21 days.

In a randomized study portal infusion combined with intraarterial infusion of 5-FU did not improve the survival compared with untreated controls (122). As has been enforced above this study includes a very limited number of patients. Improved survival has been reported with portal infusion of 5-FU compared with historical untreated patients (132).

Before definite conclusions can be drawn concerning the benefit of regional intravascular infusion of cytostatic agents, the technique has to be tested in prospective randomized studies which include untreated controls.

4. Combination of hepatic artery occlusion and regional intravascular cytostatic agent infusion

Regional intraarterial infusion of cytostatic agents to the liver in combination with hepatic artery occlusion has been reported in non-randomized studies to be superior to single treatment (133). In a prospective randomized study intravascular infusion of 5-FU alone or in combination with HAL was compared with untreated patients (122). A prolonged mean survival was observed in the patients subjected to intraportal infusion and HAL while there was no such effect seen in patients treated with HAL and intraarterial infusion of the cytostatic agent. The patients subjected to HAL and intraportal infusion received half the dose of 5-FU intraarterially.

Temporary occlusion of the hepatic artery in combination with regional intraarterial infusion of 5-FU has been tried (134). No mention has been made about which of the two procedures, temporary or permanent occlusion of the hepatic artery is the superior one in combination with intraarterial infusion of cytostatic agents.

5. Hepatic artery embolization

Interruption of the arterial blood supply to the liver is also possible by intraarterial introduction of embolic material. The effect on normal liver parenchyma with subsequent necrosis after selective hepatic artery embolization in experimental animals depends on the size and amount of embolic material. Proximal embolization or a small amount of embolic material results in no microscopic changes in the liver tissue but large amounts of distal embolization led to liver infarction which could be seen one week and up to two months after embolization (135, 136). A transient decrease in arterial liver blood flow measured with microspheres was observed after embolization and this was more pronounced after embolization with large than with small amounts of large embolic material (172 μ microspheres). Restored arterial liver blood flow was observed after one week (136). After embolization with large amounts of embolic material or distal embolization the revascularization was slower. The changes seen angiographically after embolization were restored one week up to six weeks after embolization (135, 136, 137). Embolization with degradable spheres (37 μ) also led to a transient decrease in arterial liver blood flow lasting for 30 minutes (138).

Proximal embolization (Gelfoam) and distal embolization (Silicone) induced a transient release of liver enzymes that reached a maximum value in serum within one to three days after hepatic artery embolization (135, 137). Recovery was observed one week after proximal embolization and five to six weeks after distal embolization.

Spongostan embolization of the hepatic artery in experimental animals with liver tumors induced microscopic necrosis in tumors and in normal liver parenchyma. The occlusion of the vessels lasted for one to eight days (139).

In patients with carcinoid metastases to the liver embolization of the hepatic artery with gelatin sponge revealed reduction in urinary excretion of 5-HIAA (140). Other embolic materials such as stainless steel coils have been used in humans to occlude collaterals to the liver after HAL (141).

In order to evaluate intraarterial embolization further investigations must be performed in animals and humans. A standard experimental animal model with easily detectable liver tumors at laparotomy and at angiography would probably be of great value in such studies.

6. Hepatic artery ligation and hyperthermia

Hyperthermia (a temperature exceeding 41°C) induced by radio-frequency or microwaves can be applied to the total body or locally to a tumor and can reduce tumor growth (142, 143).

The temperature range for selective destruction of malignant cells in vivo and in vitro is 41-43°C (144).

Malignant cells are claimed to be more sensitive to heat than normal ones. Heat can induce complete selective destruction of malignant cells (144, 145). Observation of no correlation between malignant potential and heat sensitivity has, on the other hand, also been reported (146).

The mechanism of the action of hyperthermia on normal and malignant cells is unknown but it induces a reversible suppression of DNA, RNA and protein synthesis, reduction in oxygen consumption, liabilization of lysosomes and an arrest or delay in mitotic activity (144, 147).

Hypoxic conditions and occlusion of the artery to, and the vein from a tumor increases the sensitivity of malignant cells to hyperthermia (148, 149, 150). It has, however, also been reported that hypoxic conditions do not increase the sensitivity to hyperthermia (151).

Hyperthermia, applied as total body or local hyperthermia with or without combination of radiotherapy or chemotherapy, has been used in clinical situations on patients with different types of malignancy (152, 153, 154). In patients with liver tumors, hyperthermia with or without a combination of chemotherapy has been reported to reduce the tumor mass in the liver (152, 155). In experimental animals a transient decrease has been shown in liver tumor volume for those subjected to hyperthermia compared with liver tumors in untreated animals (142).

The combination of HAL and hyperthermia does not appear to have been analysed. Further studies on the effect of hyperthermia on liver tumor growth in animals and humans have to be carried out before conclusions can be drawn as to how to apply hyperthermia so that it effects liver malignancy.

AIMS OF THE PRESENT STUDY

Although HAL has been the subject of clinical research for many years, its therapeutical value has not been established. This and the poor outcome for patients with liver cancer warrants an attempt to describe the effect of hepatic artery interruption on liver tumor growth in rats. This study was planned

1. to develop a method to obtain solitary liver tumors and well-defined procedures for repeated measurements of unremoved liver tumors (I),
2. to study the vascularization of a solitary adenocarcinoma in the liver by angiography and portography (II),
3. to establish which of the two methods, total liver dearterialization or hepatic artery ligation, is the most reproducible and gives the most prominent effect in reducing liver tumor growth (III),
4. to investigate if the effects on tumor growth of hepatic artery ligation (HAL) varies for different types of experimental transplanted tumors (IV),
5. to compare the effect on the reduction of liver tumor growth of hepatic artery embolization compared with hepatic artery ligation (V),
6. to test total body microwave hyperthermia in combination with hepatic artery ligation to see if the combination is more effective than HAL alone (VI).

MATERIALS AND METHODS

Animals

Sixty-eight inbred Lister-hooded rats (III, IV) and 128 inbred Wistar rats (I, II, IV, V, VI) of both sexes weighing 180-250 g were used in the experimental series (Table I). The rats were maintained on a standard rat pellet diet and water ad libitum. They were all kept free in their cages containing 5-6 rats in each. The animals subjected to total body microwave hyperthermia (VI) were during the period of hyperthermia placed in a special plexiglass cage.

Paper	Number of animals	Type of rats	Type of tumor	Passages of the tumor
I	34	W	NGW	1
II	13*	W	NGW	+34
III	20	LH	Bp	1
IV	18	LH	Bp	+12
	18	LH	Hep	1
	12	LH	Hep	+10
	12	W	NGW	+10
	12	W	NGW	+26
V	30*	W	NGW	+34, +35
VI	36	W	NGW	+44, +45

Table I. The number of passages, i.e. the generations of the three different experimental tumors and the number of rats used in the experimental series. x) indicates that nine animals are identical in these two series.

The rats were anesthetized with ether during tumor inoculation, tumor size measurements, arteriography, portography and hepatic arterial interruption. The rats were sacrificed by an overdose of ether.

Tumors

In the experimental series three different types of tumors were studied (Table I). A N-methyl-N-nitrosoguanidine-induced adenocarcinoma (NGW) in the colon was inoculated in Wistar rats (I, II, IV, V, VI), a benzopyrene-induced sarcoma (BP) (III, IV) and a hepatoma (Hep) (IV) was inoculated in Lister-hooded rats. The three tumors were propagated intrarenally every 10 days as a rough cell suspension in the appropriate strain of animals. The generation of the

three different tumors is shown in Table I. The first generation used for the present experimental series is called passage 1. The absolute number of passages before this generation were not counted but estimated to approximately 50. The condition of the tumors was controlled at regular intervals for occurrence of infection and alteration in tumor growth.

By dissecting the tumors from the kidney and cutting them into small fragments and then homogenating them by trypanization a cell suspension was achieved. The vital cells were determined by adding trypan blue (27). The unstained cells were considered to be viable and the number of stained cells was 10-15 % of the total number of cells in the suspension. A suspension containing 0.5×10^6 per 0.1 ml viable tumor cells (VI) and 1.0×10^6 per 0.1 ml viable tumor cells (I-V) was manufactured by dilution.

Through a midline incision the abdominal cavity was opened and 0.1 ml of the cell suspension was inoculated with a syringe and thin needle just under the capsule on the ventral surface of the central liver lobe. The spot where the needle perforated the capsule was approximately 5 mm from the edge of the liver lobe. To minimize leakage of tumor cells when the needle was withdrawn, a Spongostan sponge was placed over the injection spot until hemostasis was obtained. A maximum of 20 animals could be inoculated within one hour. Papers V and VI include a greater number of animals, which was made possible by inoculation with two subsequent tumor generations. In paper I the tumor was propagated in two donor animals to be used on two consecutive days for inoculation in the actual experimental animals.

Tumor size measurements

The tumors were measured with vernier calipers on the ventral surface of the central liver lobe during laparotomy. The largest (a) and the smallest (b) visible superficial diameters were recorded in mm. The tumor volume was calculated from these measurements according to different formulas (formulas 1-4) (Table II) (I). The tumors were extirpated, dissected and measured in three dimensions in the same position as they were in the liver (diameters c, d and e). The real tumor volume was calculated from these measurements according to formula 5 (I) (Table II).

Measurements of the tumor at the largest and smallest diameters on the x-ray film were used to calculate the tumor volume according to formula 3 after subtraction of the magnification of 3 % (II).

Tumor volume was calculated from measurements of two dimensions of the visible superficial largest and smallest diameters₂ of the unremoved liver tumors according to the formula $V = axb^2/2$ (II, III, IV, VI) and $V = axb (*)$ (V). All the measurements were performed

(*) Symbols, see page 18.

by one of two persons. In a separate analysis it was found that the agreement between these two persons was high ($r = 0.98$). The wet weight of the extirpated and dissected tumors were recorded in mg in all 162 animals that survived up to the end of the experimental period (I-VI).

Formula	Mean ($\pm$ SD)	Logarithmic mean ($\pm$ SD)
$V = a \times b$	57 ($\pm$ 37)	3.880 ($\pm$ 0.62)
$V = \frac{\pi}{6} \left(\frac{a+b}{2} \right)^3$	263 ($\pm$ 241)	5.197 ($\pm$ 0.94)
$V = \frac{a \times (b)^2}{2}$	219 ($\pm$ 175)	5.062 ($\pm$ 0.89)
$V = (\sqrt{a \times b})^3$	483 ($\pm$ 425)	5.820 ($\pm$ 0.93)
$V = \frac{\pi}{6} \times c \times d \times e$	223 ($\pm$ 163)	5.128 ($\pm$ 0.83)

Table II. Mean and logarithmic mean tumor volume calculated with different formulas. Symbols a-e see page 18.

Arteriography and portography

Hepatic arteriography was obtained by catheterization of the coeliac axis via the femoral artery (156). Via the superior mesenteric vein a catheter was placed in the portal vein under laparotomy and portography was performed. The films were exposed in the lateral projection. Repeated hepatic arteriography and portography were used in order to study the vascularity of solitary adenocarcinomas in the liver (II). Hepatic arteriography was used to control HAL and embolization of the hepatic artery and to follow the revascularization of the adenocarcinomas in the liver (V).

Histopathological examination

The central liver lobe and the tumor were excised, fixed in 10 % formaldehyde solution and embedded in paraffin. Hematoxylin-erythrosin stained sections ($4 \mu\text{m}$) were microscopically studied for necrosis of the tumor and normal liver tissue (V).

Hepatic arterial interruption

Through a midline incision the hepatoduodenal ligament was exposed.

The proper hepatic artery was ligated with 6.0 silk suture (HAL) (III-VI) (Fig 1). Liver dearterialization was performed using the above technique together with separation of the liver from surrounding attachments leaving the portal vein, common bile duct and the hepatic veins intact. In the sham operation the same method as liver dearterialization was used, but the silk suture was not tied (III). In the animals subjected to the control procedure only tumor size measurements were performed during laparotomy (III-VI).

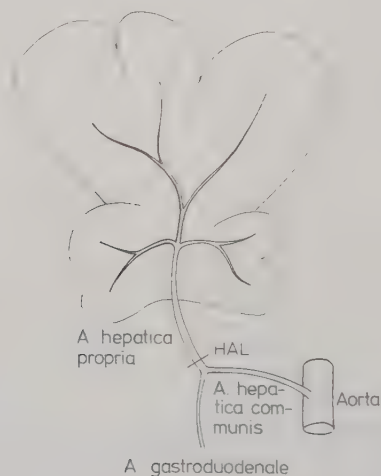


Fig 1. Schematic illustration of the anatomy of the hepatic artery in the rat and the position of the ligature.

Hepatic artery embolization

A catheter was placed in the coeliac axis via the femoral artery during fluoroscopic control. Through this catheter 0.04-0.08 ml of the embolic material was injected as a suspension of Spongostan powder (99.3 % gelatin, Ferrosan International, Denmark) mixed with contrast medium at a concentration of 0.25 mg/ml (V) (139).

Hepatic artery ligation and hyperthermia

Hepatic artery ligation (HAL) was performed as previously mentioned. During hyperthermia the rats were placed in a special plexiglass cage which prevented them from moving about. Pellets and water *ad libitum* was continued. This cage was placed in a larger plexiglass cage with a constant temperature of 25°C. A rectal probe recorded the body temperature of the rat every 30 s (VI).

Microwaves with an exposure below $4\,000\text{ W/m}^2$ generated from a Siemens magnetron (Radiotherm 305 at 2.45 GHZ max and an effect of 200W) induced the hyperthermia through an antenna placed just outside the larger plexiglass cage on the right side of the rat (Fig 2). A computerized system (INTEL 8085 microprocessor with a 16 kbyte memory) directed the microwave generator so that the rectal temperature was constant at $41.5 \pm 0.1^\circ\text{C}$. The hyperthermia was given for one hour every eight hours during 24 hours, i.e. three one-hour sessions (142).

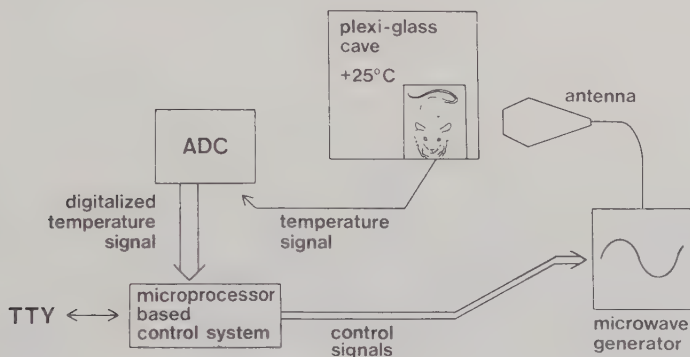


Fig 2. Experimental set-up for microwave induced total body micro-wave hyperthermia.

Statistical methods

In order to get an approximate normal distribution the data were transformed into logarithmic values. The following statistical methods were used: Student's t-test, regression analysis, analysis of variance and analysis of covariance. All tests were two-tailed.

RESULTS

The mortality rate for the experimental series is listed in Table III. Death occurred in connection with the anesthetic procedure, the x-ray procedure and during the period of hyperthermia. The cause of death has not been analysed. There were no statistically significant differences in mortality rate for the 53 animals subjected to the control procedure and the 53 animals subjected to HAL. The mortality was 5 (9 %) and 6 (11 %) animals, respectively. Eight of 24 (33 %) animals died in the groups subjected to HAL in combination with hyperthermia (VI). This figure was statistically significantly higher than the mortality rate for HAL ($p < 0.05$).

Experimental series			Days after tumor inoculation								
			5	10	12	14	17	18	21	22	24
II		n = 9		2		3			3		
III	Control	n = 5		0			0				1
	Sham procedure	n = 5		0			0				2
	HAL	n = 5		0			0				0
	Liver deart.	n = 5		0			0				1
IV	Group A	Control n = 9		0		1		1			
		HAL n = 9		0		1		3			
	Group B	Control n = 9		0			0				1
		HAL n = 9		0			0				0
	Group C	Control n = 6		0		0		2		2	
		HAL n = 6		0		0		0		2	
	Group D	Control n = 6		0			0				
		HAL n = 6		0			1				
	Group E	Control n = 6		0		0				0	
		HAL n = 6		0		0				0	
VI	Control	n = 6	0	0	0						
	HAL	n = 6	0	0	0						
	HAL + hyperthermia day 0	n = 6	0	3	3						
	HAL + hyperthermia day 1	n = 6	0	3	3						
	HAL + hyperthermia day 2	n = 6	0	1	1						
	HAL + hyperthermia day 3	n = 6	0	1	1						

Table III. Cumulative mortality in the experimental series. Symbols see paper II-IV and VI.

In none of the 196 rats were there any signs of malnutrition or infection during the experimental period. No significant changes in body weight were observed during the experimental period. Tumor take for the three different tumors was 100 %.

No macroscopic signs of infection in the tumors, metastatic growth or tumor overgrowth on neighbouring organs were observed. All tumors were at laparotomy, angiography, portography and autopsy, solitary and localized in the periphery of the central liver lobe. The visible superficial surface of the tumors as well as the visible tumor mass protuberant over the liver surface were independent of size and tumor type, all ellipsoid in shape at laparotomy. The shape of the visible superficial part of the unremoved liver tumors was in good agreement with the shape in all three dimensions of 161 extirpated and dissected tumors (I-VI). In one rat the visible superficial shape was incongruent with the real shape of the extirpated and dissected tumor (I).

A comparison of the tumor volume calculated from measurements of the two visible superficial diameters of the unremoved adenocarcinomas in the liver with the tumor volume calculated from measurements in three dimensions of extirpated and dissected tumors and the tumor weight indicates that formula $V_3 = a \times b^2/2$ (*) was the best choice for tumor volumes between 15 mm³ to 710 mm³ (correlation coefficient $r = 0.88$). The optimal formula for estimating tumor volume of the unremoved NGW tumors was $V = a^{1.05} \times b^{1.62} \times 0.999$ (*).

The tumor volume₂ (121-4273 mm³) of the unremoved sarcomas using formula $V = a \times b^2/2$ (*) compared with the weight of the extirpated and dissected sarcomas gave a correlation coefficient $r = 0.97$. The same analyses for the hepatomas (208₃-9626 mm³) gave $r = 0.98$ and for all adenocarcinomas (42-2465 mm³) $r = 0.94$ except those in paper I.

The outline of the adenocarcinomas subjected to repeated hepatic arteriography or the portography was ellipsoid in the lateral projection on the film. This outline was not influenced by increasing tumor size (II and V). Calculation of the tumor volume at laparotomy using formula $V = a \times b^2/2$ (*) shows no statistically significant difference when compared with the tumor volume calculated from measurements on the x-ray film after arteriography using the same formula (II).

The arterial vascular pattern of the adenocarcinomas in the liver varied depending on tumor size. Adenocarcinomas with a diameter of 3-8 mm showed a hypovascularized central part and a hypervascularized periphery (Fig 3). With increasing tumor size (diameter 5-12 mm) the tumor feeding artery increased in size and the vasculariza-

(*) Symbols, see page 18.

tion of the adenocarcinomas was more homogeneous. No pronounced border between central and peripheral parts of the tumors was observed. The central part of the adenocarcinomas then became hypovascularized again with increasing tumor size (diameter 8-20 mm). The arterial vessels leading to the peripheral parts of the adenocarcinomas had increased in size.

The portography revealed portal vessels mainly in the peripheral part of the adenocarcinomas. Portal vessels were also found leading to the central part of the tumors with contrast loading of the tumor (II) (Fig 3).

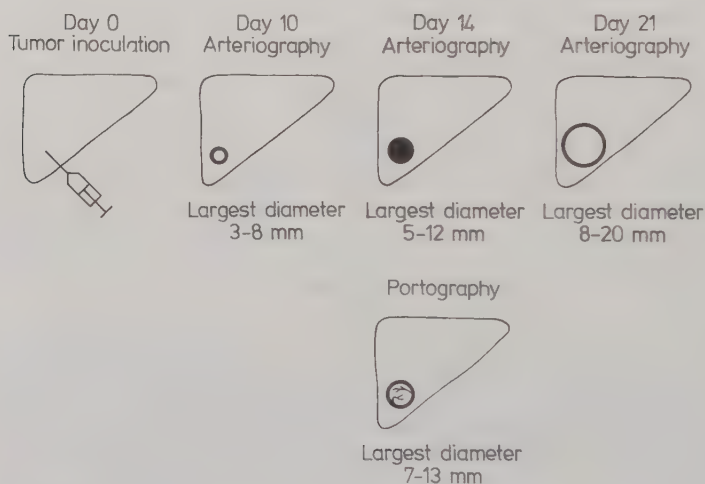


Fig 3. Schematic illustration of tumor size and variations in tumor vascularity. Vessels are indicated with black.

No statistically significant differences in tumor volume between the HAL animals and the controls were observed at the start of the experimental period.

In all control animals the three experimental tumors used showed a continuously increasing tumor volume during the experimental period. In the animals subjected to HAL there was a reduction in tumor growth during the experimental period except for the series with the large hepatomas (volume 256-684 mm³) and large adenocarcinomas (volume 128-206 mm³) (III, IV, V, VI).

The growth of the sarcomas was not reduced by regular liver dearterialization. In the animals subjected to sham procedure or regular liver dearterialization there was a statistically significant faster tumor growth than in the controls (III).

Hepatic artery ligation (HAL) reduced the growth of the sarcomas during the experimental period. The induced reduction in tumor growth of the sarcomas after HAL was not influenced by the tumor generation (III, IV) (Fig 4).

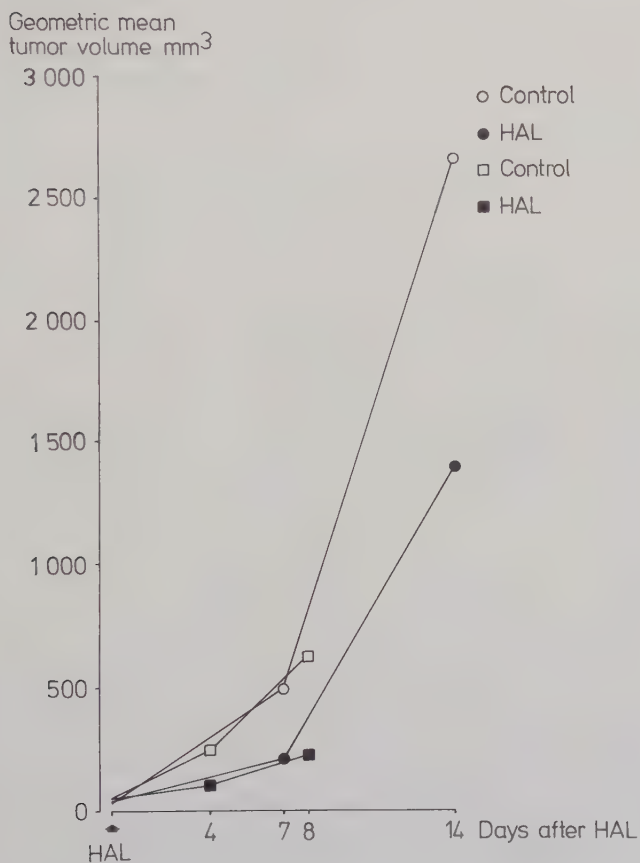


Fig 4. Geometric mean tumor volume for the animals inoculated with the benzpyrene-induced sarcoma. Circles indicate the tumor of the first generation (III) and squares indicate a tumor 12 generations later (IV).

The growth of the hepatomas was influenced by the generation of the tumor with a statistically significant smaller liver tumor volume obtained with the ten generation older tumor at the time for HAL ($p_3 < 0.01$) (Table I, Fig 5). For the small hepatomas (volume 62-121 mm^3) HAL induced four days later a statistically significant decrease in tumor volume ($p < 0.002$) (Fig 5). During the experimental period HAL induced a slower tumor growth that was statistically significant. The growth of the large hepatomas (256-684 mm^3) was not influenced by HAL (Fig 5).

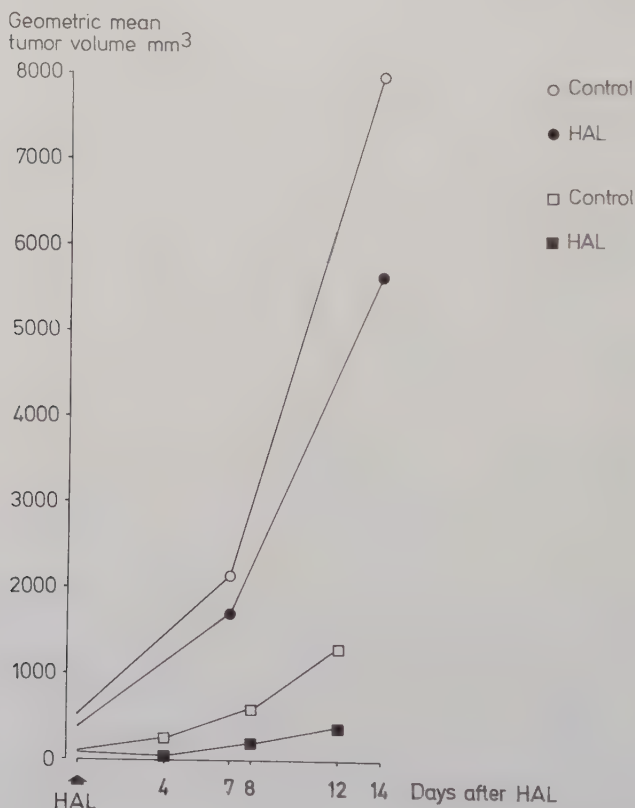


Fig 5. Geometric mean tumor volume for the animals inoculated with the hepatoma. Circles indicate the tumor of the first generation (IV) and squares indicate the tumor 10 generations later (IV).

The growth of the adenocarcinomas was influenced by the generation of the tumor. At the time for HAL the volume was significantly larger in the young tumor ($p < 0.01$) (Table I, Fig 6). The influence of tumor generation on the growth disappeared with increasing passages.

A statistically significant decrease in tumor volume was observed four days after HAL in two series with small adenocarcinomas ($p < 0.001$) (Fig 6) (IV, V). The induced reduction in tumor growth of the small adenocarcinomas of HAL was observed during the observation period. In one further series a transient reduction in tumor growth rate compared with controls was observed (Fig 6) (V).

The growth of large adenocarcinomas was not influenced by HAL during the experimental period (Fig 6) (IV).

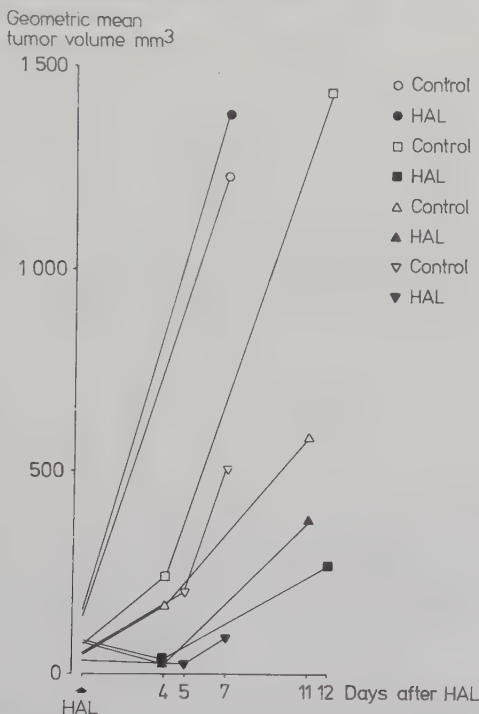


Fig 6. Geometric mean tumor volume for the animals inoculated with the adenocarcinoma. Circles indicate the tumor of the 10th generation (IV), squares indicate a tumor of the 26th generation (IV), triangles with the right side up indicate a tumor of the 34th and 35th generation (V) and triangles up side down indicate a tumor of the 44th and 45th generation (VI).

The adenocarcinomas showed four days after occlusion of the hepatic artery by embolization with Spongostan a transient reduction in liver tumor growth (V). The reduction in tumor growth of the adenocarcinomas induced by embolization was not so prominent as that induced by HAL ($p < 0.05$)

The growth of small adenocarcinomas was not further reduced by the addition of total body microwave hyperthermia two days after HAL (VI). During the experimental period the tumor volume in the animals subjected to hyperthermia three days after HAL was statistically significantly larger than the tumor volume in the animals subjected solely to HAL.

HAL occlusion was controlled by arteriography in six animals and was found to be complete. In five of six animals Spongostan embolization induced total occlusion of the hepatic artery (V).

Within 15 minutes after HAL or embolization, revascularization of the liver by pre-existing collaterals in the liver hilum was observed. At that time no vessels to the tumors was seen. Four days later, the liver and the tumor were found to be revascularized by collaterals after HAL and after Spongostan embolization by collaterals and recanalization of embolized vessels (V).

In rats with NGW tumors subjected to HAL or Spongostan embolization as well as in controls, tumors showed extensive central necrosis with a peripheral rim of preserved vital tumor tissue 11 days after therapeutic procedures. No difference between the groups concerning extent of necrosis was found (V).

DISCUSSION

The rat is a suitable experimental animal for the study of hepatic artery interruption since the proper hepatic artery is easily identified and ligated (Fig 1) and the rat can sustain HAL. After ligation of the hepatic artery in rats not subjected to repeated laparotomies no mortality was registered (98). In the present study it seems reasonable to believe that the observed mortality rate of approximately 10 % after HAL is due to the repeated laparotomies. This may be borne out by the absence of any difference in mortality in the animals subjected to the control procedure and the HAL procedure.

Moreover, techniques for hepatic arteriography and hepatic artery embolization are available for rats (139, 156). Total body microwave hyperthermia has been tested and optimal temperature and duration established in rats with liver tumors (141). In inbred rat strains experimental liver tumors characterized according to tumor take, tumor growth, vascular pattern and tumor blood flow are available (20, 24, 25, 30).

The disadvantages of using the rat primarily rest in the problem of applying the experimental findings to the clinic.

In order to get a broader analysis the influence of hepatic artery interruption on liver tumor growth was tested on experimental tumors of three different types.

These three transplantable tumors had a somewhat different growth rate. The hepatoma had the highest growth rate while the adenocarcinoma had the lowest. The growth rate of the hepatoma and the adenocarcinoma was influenced by the generation of the tumor (Table I) (Figs 5 and 6). This is in good agreement with the observations made by other authors (33, 34).

The three different tumors when growing in the liver have been previously characterized according to their arterial blood flow (25). The relative arterial tumor blood flow decreased with increasing tumor weight for all three tumor types. The largest extirpated and dissected tumor in the present study was $17.0 \times 11.4 \times 7.0$ mm, i.e. the actual tumor volume was 710 mm^3 which corresponds to a tumor weight of 0.78 g (I).

Only in one series (Hep IV) some tumors exceeded to some extent this size at the time for hepatic artery interruption. Based on the findings by Sundqvist et al it can be postulated that the liver tumors used in the present investigation were at the time for HAL well nourished with arterial blood flow of more than 0.4 ml/min/g (157).

In a pre-experimental series, Lister-hooded and Wistar rats carrying the experimental liver tumors survived approximately 40

days before there were signs of malnutrition, metastases and infection preceding the death of the animal a few days later. In all experiments the animals were sacrificed no later than 24 days after tumor inoculation, i.e. before they showed signs of malnutrition etc. The documented absence of infection or metastatic growth and no observed changes in body weight during the experimental period confirm that this margin was sufficient.

In some experimental series (V, VI) the number of animals exceeded the maximum of 20 that could be inoculated within one hour after preparation of the tumor cell suspension. This higher number of tumor-carrying animals was obtained with one tumor generation between the inoculations. The animals subjected to this extra procedure were evenly distributed between control and experimental procedures. The animals were included in the statistical analyses only if the tumor volume was of a similar size at the start of the experimental period and if the changes in tumor volume for the control series were similar for the two tumor generations during the experimental period.

Administration of hepatocarcinogenic substances to rats induces not only liver tumors but also liver cirrhosis (22). Portal injection of viable tumor cells in order to induce liver tumors may cause portal thrombosis or obstruction of portal vessels by tumor masses.

Liver cirrhosis and obstructed portal vessels decrease the portal blood flow which in its turn may lead to an increased hepatic arterial blood flow. In humans and in experimental animals an increased blood flow through the hepatic artery is observed after occlusion of the portal vein (78, 79, 80). Apart from the obvious disadvantages of a scattered liver tumor growth after administration of hepatocarcinogenic substances or portal injection of tumor cells, the speculated influence on the portal circulation and subsequent changes in hepatic arterial blood flow disqualifies these techniques for inducing liver tumor in the present investigations.

The technique of inducing the liver tumors by inoculation of a defined tumor cell suspension directly into the liver parenchyma was highly reproducible and gave a solitary well defined ellipsoid liver tumor (I-VI).

It was established that several formulas gave a good estimation of the tumor volume of the unremoved adenocarcinoma in the liver. However, calculation from measurements either at laparotomy or angiography in two dimensions using the formula $V = a \times b^2/2$ (*) gives the best correlation to the actual volume and/or the tumor weight of the extirpated and dissected tumor (I-VI).

(*) Symbols, see page 18.

The radiological investigations in living animals led to two interesting findings: the occurrence of the portal vessels in the central part of the adenocarcinomas and the occurrence of a transient homogeneous arterial vascularization for the adenocarcinoma with a diameter of 5-12 mm (volume 62-364 mm³) (II) (Fig 3). These findings are, however, based on a restricted number of observations and only on the N-methyl-N-nitrosoguanidine-induced adenocarcinoma.

Injection of dye solutions and portography of tumors induced by hepatocarcinogenic substances showed that there were portal vessels within hepatocellular carcinoma but cholangiocellular carcinoma had no portal vessels in the tumor (20, 59). This difference in vascularization between the hepatocellular carcinoma and cholangiocellular carcinoma may have been due to concomitant liver cirrhosis, though this was not mentioned. In another study with liver tumors induced by the same hepatocarcinogenic substance, liver cirrhosis was found (22). In the present study, however, there were no macroscopic signs of liver cirrhosis. It must also be observed that Honjo and Matsumura with injection of dye solutions identified portal vessels sporadically within a Walker tumor induced by portal injection of tumor cells (59).

If the findings of intratumoral portal vessels and a homogeneous arterial vascularization in liver tumors can be applied to human liver cancer this may have implications for the indications to carry out hepatic artery ligation and intravascular cytostatic agent infusion.

In rats ligation of the proper hepatic artery induced a slower liver tumor growth in three different types of experimental tumors up to 14 days after the procedure (III-VI). Even a decreased tumor volume was observed in some experiments on the fourth day after HAL.

In two series with somewhat larger hepatomas and adenocarcinomas, respectively, at the time for HAL no effect was registered (IV). This finding can possibly be explained by a larger content of anaerobic cell clones in the larger tumor. These clones may be insensitive to or even stimulated by an anoxic-hypoxic situation.

Spongostan embolization of the hepatic artery also resulted in reduced, though transient liver tumor growth of adenocarcinomas (V).

It must, therefore, be concluded that hepatic artery interruption (HAL) can decrease the size and retard the growth rate of different experimental liver tumors. These findings have not been established before but there are findings though not statistically analysed of a prolonged survival after HAL in rats with two different sarcomas (90).

The effect of HAL in clinical series varies considerably (158). In patients with colorectal metastases to the liver a prolonged survival has been found after HAL alone or with intraportal or intra-arterial cytostatic agent infusion as compared to historical controls (132, 133, 159). The results have not been analysed to any great extent according to tumor type or size. The magnitude of liver tumor at the time for HAL can be one explanation for the varying effect.

It is a matter of speculation whether the effect of HAL is better on eight small liver tumors each with a diameter of approximately 7 mm and a total volume of 1 370 mm³ than on one tumor with the same volume (approximate diameter 14 mm).

As has been indicated before the only randomized clinical trial so far executed has not shown any significant prolonged survival after HAL (122). The patients in that group were also treated with small amounts of intraarterial 5-FU infusion. The combination of HAL and 5-FU intraportally and intraarterially showed, however, prolonged survival. In this study by Taylor (122) the results were based on a restricted number of patients. Neither were the patients stratified according to the tumor size and the arterial and portal vascular pattern of the liver tumors at the time for ligation of the hepatic artery.

In patients with colorectal metastases in the liver, tumor vascularity was found to be a prognostic factor (56). A better arterial tumor vascularization at the time for HAL and intravascular cytostatic agent infusion resulted in a prolonged survival. The carcinoid tumor in the liver is considered to be particularly sensitive for HAL. This tumor is also very vascularized.

The relationship between tumor size and the varying vascular pattern, both arterial as well as portal as we found (II) and the relative tumor blood flow as found by Sundqvist et al (25, 157) should give hints when hepatic artery occlusion could be expected to give the most pronounced effect.

Further studies have to be performed in both experimental and clinical series in order to establish the effect of HAL on liver tumor growth in relation to the vascular pattern and tumor size at the time for HAL. The clinical studies have to be stratified for the size of the liver tumors and their vascularity.

The present study also emphasizes the importance of the timing of additive therapeutical procedures to HAL as its maximum effect has disappeared already after four to five days. This finding is supported by Ackerman who found a recovered tumor blood flow four days after HAL (88).

Further support for the shortlasting effect of HAL is the revascularization of the liver by collaterals in the hilum which was

observed within 15 minutes after occlusion of the hepatic artery either by ligation or Spongostan embolization (V). This observation is in good agreement with other experimental studies (93, 95). In humans the arterial circulation was found to be restored after four days (71, 94). It is possible that the recovered arterial vascular bed to the liver contributes to the transient effect of HAL.

Liver dearterialization, i.e. interruption of all arterial blood supply to the liver was believed to be more effective in inducing tumor necrosis since HAL in rats and humans did not cause total tumor necrosis (97, 98). The rapid revascularization of the liver after HAL supported the assumption of superiority of liver dearterialization (94). However, clinical experience has failed to show that this extensive surgical procedure is more effective in the control of liver tumor growth than only ligation of the proper hepatic artery (118, 159). Clinical experience is supported by the present experimental study. In fact in a very restricted number of animals it was shown that HAL was better in reducing the tumor growth rate than regular liver dearterialization (III). As HAL is a less extensive procedure in the experimental animal and the patient, this technique so far should be the accepted one until results from prospective clinical trials prove otherwise.

A temporary occlusion of the hepatic artery has been tested and given clinical results similar to those from permanent occlusion (121, 123, 134). Temporary occlusion may cause reduced stimulation to opening of collaterals although in experiments in humans the opening of collaterals was incident (95). Other means to reduce the effects of collateralization would be to occlude the collaterals with embolic material (141).

In humans embolization of the hepatic artery has been found to reduce liver tumor growth (140, 160). Embolization of the hepatic arterial vessels ought to be evaluated in prospective clinical trials since the advantage of this method is that it enables repeated embolization without the need of an operation.

Total body microwave hyperthermia induced a transient short-lasting reduced growth rate of liver tumors (142). In the present study total body microwave hyperthermia in combination with HAL did not reveal an additive or synergistic effect (VI). In fact hyperthermia added three days after HAL seemed to stimulate tumor growth. This increased tumor growth may be an illusion related to an oedema in and around the tumor which gives false estimations of tumor volume. Hyperthermia added the same day and one day after HAL was accompanied by pronounced mortality (50 %). Mortality after total body microwave hyperthermia of the same temperature and duration was 30 % (142).

If homeostasis and liver function can be controlled and mortality reduced, the combination of HAL and total body hyperthermia may be more effective. Another possibility to reduce the mortality of the

combination of HAL and hyperthermia may be to induce hyperthermia by local application to the liver or the tumor alone.

It is at this point appropriate to point out that hypoxia has not yet been proved to be the mechanism behind the effect of HAL. Other factors may be of importance, i.e. lack of glucose or other substrates for the nutrition of the tumor cells. The rapid glycolytic activity in malignant tumors is capable of maintaining the ATP concentration in the tumors better than in normal liver tissue during a total ischemic period of the liver for at least 10 minutes (161). Without an increased employment of oxygen from the portal blood (5) this may imply that total interruption of hepatic arterial blood flow may damage normal liver parenchyma more than the tumor.

There are probably also cell clones in a malignant tumor which almost only engage an anaerobic metabolism. In an anoxic or hypoxic situation as expected after HAL such cells will be stimulated to an increased mitotic activity. An investigation on the variation in oxygen consumption of different tumors seems to be important in order to identify tumor types that are most sensitive to HAL.

GENERAL SUMMARY AND CONCLUSIONS

1. This investigation introduced a model to obtain solitary easily measurable liver tumors in rats. The tumor had a ellipsoid shape. The tumor volume could be calculated under laparotomy from the two visible diameters. The best formula was $V = a \times b^2/2$ (*).
2. The vascularity of the adenocarcinoma could be studied by angiography and portography. There was a variation in the arterial vascularity related to tumor size. Tumor volume could be calculated according to the same formula $V = a \times b^2/2$ (*) for measurements on the x-ray film.
3. HAL was a better procedure than regular liver dearterialization to decrease the growth rate of experimental liver sarcoma.
4. The observed effect of HAL was reduced tumor growth indicated by a decrease or a slower increase in tumor volume compared with controls. The effect was most pronounced for 4-5 days but was maintained for at least 14 days in several series. HAL had an effect on sarcomas, hepatomas and adenocarcinomas. The tumor size at the time for HAL seemed to have an influence.
5. Intraarterial Spongostan embolization gave results similar to those after HAL, although the effect was not so pronounced.
6. The combination of hyperthermia and HAL had no additive effect in reducing liver tumor growth compared with only HAL.

(*) Symbols, see page 18.

FUTURE PROSPECTS

Hepatic artery occlusion has been employed in clinical work for more than 15 years. As a single procedure its value is doubtful or more than doubtful for liver tumor control.

It has been claimed that liver dearterialization is more effective than HAL and that temporary occlusion of the hepatic artery is superior to permanent occlusion (158). This has, however, not been borne out by clinical studies. Permanent occlusion of the hepatic artery in combination with intraarterial and intraportal infusion of 5-FU has shown a prolonged mean survival.

The future in this field involves further phase-III studies to establish the value of HAL in combination with different types of cytostatic agents. It also includes properly designed phase-I and phase-II studies evaluating intermittent hepatic artery occlusion with different devices such as intraarterial balloon catheter, intraarterial embolization with degradable microspheres, polyethylene slings placed around the hepatic artery or external mechanical devices.

It is quite clear that further animal experiments are necessary for a thorough investigation of the effect on liver tumor growth of the pathophysiological process induced by HAL alone or in combination with additive procedures.

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